Parasitology International 61 (2012) 38–45

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

The ecology of the *Bithynia* first intermediate hosts of *Opisthorchis viverrini*

Trevor Petney [a,*], Paiboon Sithithaworn [b,c,**], Ross Andrews [b,c], Nadda Kiatsopit [b,c], Smarn Tesana [b,c], Carl Grundy-Warr [d], Alan Ziegler [d]

[a] Department of Ecology and Parasitology, Karlsruhe Institute of Technology, Kornblumenstrasse 13, Karlsruhe, Germany
[b] Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[c] Liver Fluke and Cholangiocarcinoma Research Center (LFCRC), Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[d] Department of Geography, National University of Singapore, 21 Lower Kent Ridge Road, Singapore 119077

ARTICLE INFO

Article history:
Received 28 February 2011
Received in revised form 14 July 2011
Accepted 22 July 2011
Available online 29 July 2011

Keywords:
Bithynia siamensis goniomphalos
Bithynia funiculata
Epidemiology
Cercarial prevalence
Habitat

ABSTRACT

Opisthorchiasis, together with its associated cholangiocarcinoma, is one of the most important human parasitic diseases on continental Southeast Asia. A great deal of epidemiological data from humans is available on this disease, particularly from the northeast of Thailand, however, only limited information is available on those aspects of the life cycle relating to its *Bithynia* (Gastropoda) and cyprinid fish intermediate hosts. Here we review the information which is available on the *Bithynia* hosts of *Opisthorchis viverrini*. Only one major ecological study has been carried out at one site on a single species of *Bithynia*. We show not only that detailed ecological studies are required to clarify the epidemiology of opisthorchiasis, but also that the taxonomic status of the *Bithynia* species transmitting *O. viverrini* requires clarification.

1. Introduction

The trematode *Opisthorchis viverrini* is one of the most important human pathogens in the middle and lower Mekong area, comprising the north and northeast of Thailand, Lao PDR, Cambodia and southern Vietnam, where an estimated 67.3 million people are at risk of infection [1]. Incomplete records from Thailand and Lao PDR indicate that over 9 million people are infected, but as Thailand is the only one of these countries with an adequate disease registration system this number is likely to increase substantially as more information on prevalence, particularly from Cambodia and southern Vietnam, becomes available [1].

O. viverrini has a complex life cycle (Fig. 1.) with eggs shed mainly by human hosts washing into freshwater habitats occupied by snail first intermediate hosts belonging to the genus *Bithynia*. These snails eat the eggs and become infected. On maturity, the cercariae are released into the water where they actively seek a fish host belonging to the family Cyprinidae [2]. Once in these second intermediate hosts, the developmental cycle progresses to the metacercarial stage, after which potential human and carnivore hosts become infected when they eat the infected fish. Human infection occurs when raw, fermented or marinated fish, not adequately heated, is eaten [1]. Infection is common within the Mekong area as such fish dishes make up a traditional part of the diet [3].

* Corresponding author. Tel./fax: +49 721 60843913.
** Correspondence to: P. Sithithaworn, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. Tel.: +66 43 348387; fax: +66 43 202475.
E-mail addresses: Petney@kit.edu (T. Petney), paib_sit@hotmail.com (P. Sithithaworn).

1383-5769/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2011.07.019

Although the life cycle of *O. viverrini* has been known for many decades, few detailed studies have been carried out on its first and second intermediate hosts. Many of these have been published in local journals and are therefore difficult to access. In addition, recent work on the genetic structure of geographically separated populations of *O. viverrini*, as well as of its *Bithynia* first intermediate hosts suggests that both represent species complexes [4]. This work implies that any ecological study of the parasite or its hosts must take into account the geographic origin of the samples [1]. Here, we provide a review of the ecology of the *Bithynia* species which are involved in the life cycle of *O. viverrini*.

2. *Bithynia* taxa identified along the Lower Mekong?

Members of the genus *Bithynia* are known from fossil deposits in the Mae Moh Basin, northern Thailand dating back to the Middle Miocene some 13 million years ago [5,6]. Today, three *Bithynia* taxa are recognized from the Mekong area as first intermediate hosts of *O. viverrini*: *Bithynia funiculata* from the north of Thailand, *B. siamensis goniomphalos* from Lao PDR and northeast Thailand to southern Vietnam including the Tonle Sap in Cambodia, and *B. s. siamensis* from western and central Thailand, south of Mandalay in Myanmar, the Malaccan Peninsula, and south of the distribution of *B. s. goniomphalos* in Cambodia and southern Vietnam [7–9]. Another species, *Bithynia pulchella*, ranging from India to the north of Thailand has not been associated with *O. viverrini* [7].

The Lower Mekong River in Thailand, Lao PDR and Cambodia is recognized as a biodiversity hotspot for gastropod mollusks with ca.

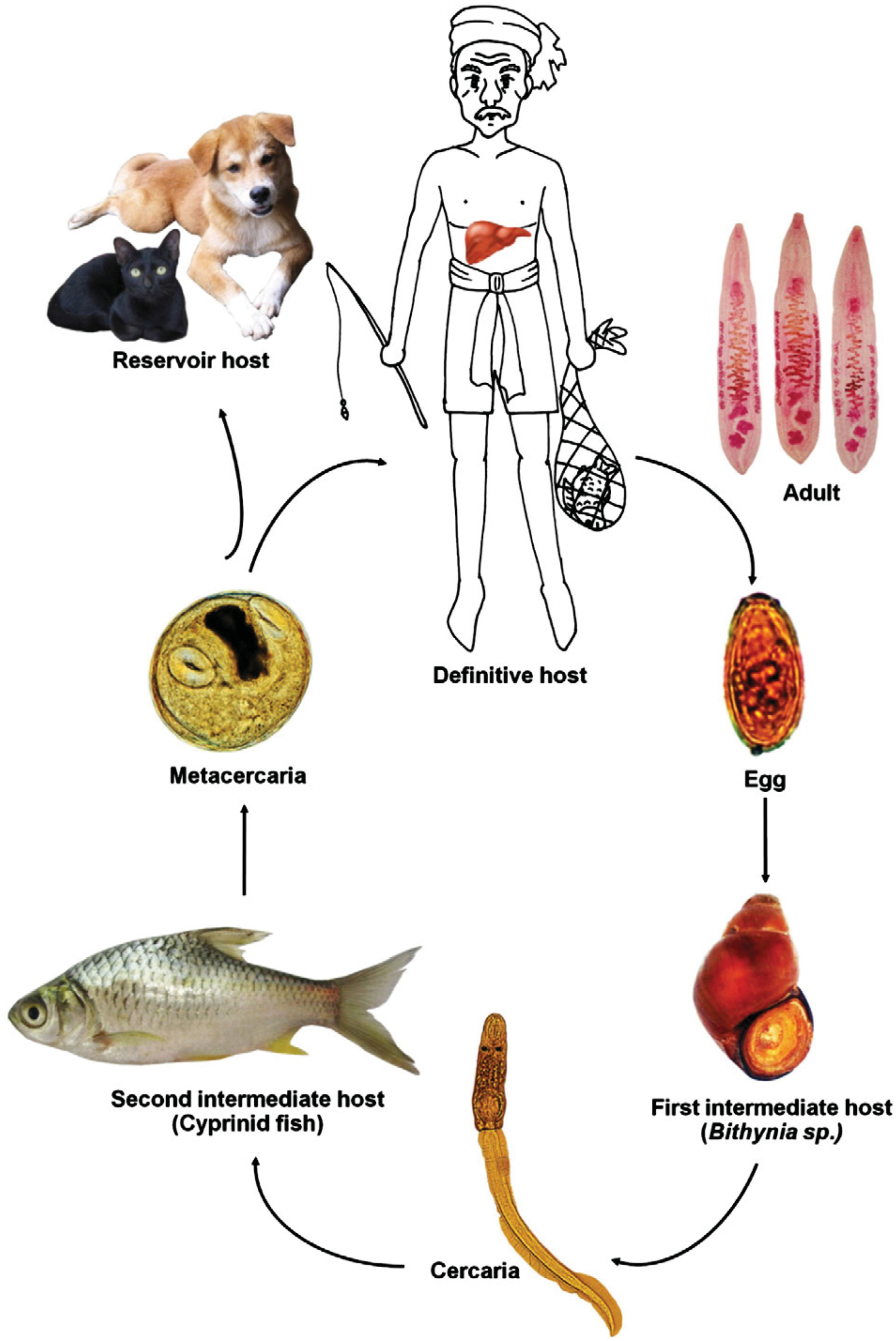

Fig. 1. The life cycle of *Opisthorchis viverrini*.

111 recognized endemic species [10,11]. Davis [10] points out that gastropod species often have abutting or barely disjunct demes probably with little or no gene flow between them.

> "Considering the habitat complexity and width of the 300 mile stretch of river that also includes major tributaries, and the patchiness and small area of species and deme patches throughout this area, it is highly probable that speciation has taken place in this centre as habitats evolved and diversity increased." [10].

This is an ongoing process.

Of the ca. 130 species in the genus *Bithynia* worldwide, about 25 occur in the Oriental Region [11]. Brandt [7] recognized two subgenera from Thailand, *Gabbia* and *Digoniostoma*, containing 3 species each, with *Bithynia* (*Digoniostoma*) *siamensis* containing 2 subspecies *B. s. siamensis* and *B. s. goniomphalos*. *Gabbia* has now been raised to generic status [12,13]. Brandt [7] indicated that future studies would be likely to alter this taxonomic situation as many species in the family Bithyniidae were still anatomically unknown. Chitramvong [14,15] has now provided more detailed anatomical and morphological analyses, although only a single sample (population) was used for each species.

Recently, a number of new species of *Bithynia* have been described elsewhere, with the authors suggesting that the genus is more diverse than previously realized [16–18]. This can be related to their low ability to disperse and small species ranges [19], fitting closely Davis' pattern for gastropod speciation along the Mekong.

The *Bithynia* taxa which act as intermediate hosts of *O. viverrini* have all been characterized, and are identified, on the basis of morphological

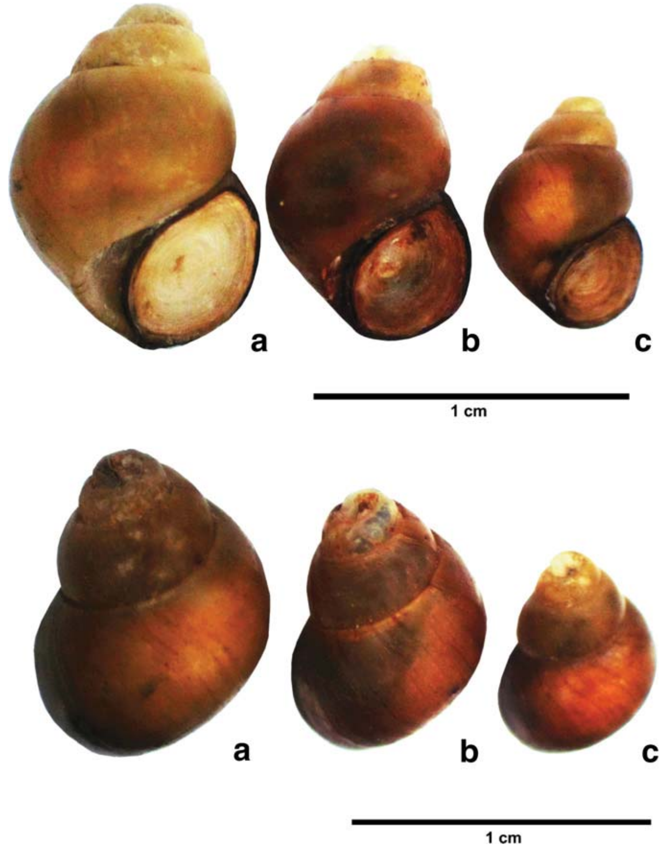

Fig. 2. Snail host of *O. viverrini* in Southeast Asia. a. *Bithynia funiculata*. b. *B. siamensis goniomphalos*. c. *B. s. simensis*.

characteristics [7,15] (Fig. 2). A recent molecular taxonomic study, however, has shown that *B. s. goniomphalos* from the northeast of Thailand and Lao PDR show considerable genetic variability with populations from different wetlands being genetically distinct [4]. The degree of genetic differentiation suggests that at least 2 species are present. In a follow-up study using multilocus enzyme electrophoresis, substantial genetic differences were found not only between *B. funiculata* and *B. siamensis*, but also between the two subspecies of *B. siamensis.* Fixed genetic differences were detected at 67–73% of the 13 diagnostically useful loci among these taxa which in turn differed from a closely related species, *Hydrobioides nassa,* at 88% of loci. A total of 73% fixed genetic differences were detected between *B. funiculata* and the two sub-species *B. s. siamensis and B. s goniomphalos*. Our data reveals similarly large genetic divergence, 67% fixed genetic differences, between *B. s. siamensis* and *B. s. goniomphalos,* which may well represent different species rather than subspecies as currently defined [20]. Interestingly, work on other gastropods has shown that morphological characteristics are inadequate as a means of differentiating species [21], although even classical morphological methods have recently been used to define new *Bithynia* species and to associate these with individual lakes and drainage systems in the Balkans [17,18]. In Thailand, evidence is accumulating from other gastropod species that there is restricted gene flow between drainage systems. For example, *Mekongia sphaericula extensa* is confined to the Songkram River and *M. s. sphaericula* to the Mun River [22]. This fits a similar pattern to the local endemism of rissoacean prosobranch gastropods other than *Bithynia* spp. The genus *Stenothyra*, is postulated to have speciated following the isolation of populations in the various tributaries and island regions of the Mekong River system [23].

These studies suggest that there is a case for considering the likelihood of a larger complex of morphologically similar species making up the intermediate hosts of *O. viverrini*. As the genetically distinguishable populations of the intermediate hosts of *O. viverrini* occur in different wetland systems, an analysis of ecological patterns must take the origin of specimens into account.

3. Recent distribution records of the *Bithynia* intermediate hosts of *O. viverrini* in Thailand

Recent work has shown the almost exclusive presence of *B. funiculata* in the north of Thailand, *B. s. siamensis* in the central area and *B. s. goniomphalos* in the northeast (Table 1). This mirrors the regions sampled with most research being concentrated in areas already well characterized. The major exception to this scheme is the seminal paper on the mollusks of Thailand by Brandt [7] which shows a much broader distribution for *B. s. siamensis* including northern, western and southeastern Thailand with considerable areas of sympatry with *B. funiculata*, with which it was collected. This strongly suggests that the selective sampling of locality carried out to date provides biased information at least for *B. s. siamensis*.

4. Habitat choice

The Bithyniidae are generally dwellers of slow flowing, muddy rivers, artificial and natural ponds, lakes, irrigation canals and swamps [24]. *Bithynia s. goniomphalos* is no exception, occupying a wide variety of such habitats including temporary and permanent freshwater bodies, shallow ponds, marshes, reservoirs and irrigation canals [7,14] (Fig. 3). They can be found on and in the mud, on rocks as well as on vegetation [25]. In Lao PDR, *B. s. goniomphalos* appears to be the most common species in paddy fields [26] and reservoirs, while *B. s. siamensis* is more commonly found in irrigation canals and drainage ditches with flowing water and artificial ponds [26]. Chitramvong [15] indicates that *B. s. siamensis* prefers a substrate of algae or other water plants such as lotus leaves. Giboda et al. [26] indicate that *B. s. laevis*, which is considered to be a synonym of *B. s. siamensis* by Brandt [7], is an “ecological form” of *B. s. goniomphalos* found in running water where it occurs together with *B. s. siamensis* in such habitats [27]. Thammasiri et al. [28] mention unpublished data showing that *B. s. goniomphalos* can occur at a depth of 3 m, while Tesana [29] indicates that it is found at all depths up to 10 m, but not at depths below 12 m. Chitramvong [14] indicates that *B. funiculata* prefers shallow water no more than about 45 cm deep even in the monsoon season.

In a recent study on *B. funiculata* in northern Thailand, this species was continuously present in paddy fields with loose soil and clay, sporadically present in an area with clay soil and not present in irrigation canals or in paddy fields with sandy and clay soils. There was no significant correlation between number collected per month and monthly rainfall, although sampling was only carried out over 5 months which resulted in a very low sample size [30]. This species was present in water with a temperature range between 24.5 °C and 31.8 °C, dissolved oxygen (DO) 2.03 to 7.66 mg/l, DO% 26.7–95.0, conductivity recorded as being between 0.000 and 0.264 mS/cm, turbidity between 16 and 288 nephelometric turbidity units (NTU), and a pH of between 6.58 and 7.56 [30]. The presence of *B. funiculata* was positively associated with areas of higher turbidity and lower pH. Although Ngern-klun et al. [30] found strong habitat similarity between *B. s. goniomphalos* and *B. funiculata*, including the former not being found below a water depth of 30 cm in the north of Thailand, this contradicts data from the northeast of the country where *B. s. goniomphalos* was found at depths of well over 1.5 m [25,29]. The most detailed study of the habitat characteristics of *B. s. goniomphalos* was carried out by Lohachit [24] in Khon Kaen province between June 1989 and May 1990. In general, the results show that this subspecies can exist in a wide range of environmental conditions. It was found at water temperatures from 18 °C to 33 °C, turbidities from 8 to 450 FTU, pH from 6.3 to 8.5 and dissolved oxygen from 2–10 mg/l. Lohachit [24] suggests that maximum

Fig. 3. Typical habitat occupied by *Bithynia siamensis goniomphalos*. a. Irrigation canal. b. Rice paddy field.

and minimum values for pH may be important limiting factors; density was highest in only slightly acid or alkaline conditions.

5. Dispersion patterns, abundance and gastropod community structure

Each of the three *Bithynia* taxa which are known to be intermediate hosts of *O. viverrini* are widely distributed with imprecisely defined but at least mostly allopatric distributions [9]. In at least some areas of Thailand they are the dominant gastropod species [24,31,32]. Lohachit [24], for example, found that 84.7% of all snails (11 species and subspecies) collected at Na Lom, Khon Kaen Province were *B. s. goniomphalos*, in a resettlement village in the same Province he found 65.5% (14 species and subspecies), while at Non Swan (Khon Kaen Province) the total was 92.5% (16 species and subspecies).

In a survey of 4 study areas around Chang Mai in the north of Thailand, Ngern-klun et al. [30] recorded at least 15 species of freshwater snails from 7 families. Of the 2240 specimens, 50 or more were found in 10 of the 15 species. *Bithynia funiculata* ranked third in overall abundance with a total of 352 (15.7%) specimens. A detailed study of the mollusks from Khon Kaen Province showed that *B. s. goniomphalos* was the most common species at 8 of 12 collection sites and was the most common species overall, representing 40.5% of all mollusks that were found [32]. There was a small but significant negative correlation ($r = -0.182$, $P < 0.05$) between the density of *B. s. goniomphalos* and that of *Filopaludina m. martensi* (Viviparidae), which may be a food competitor [32]. At the Ubolratana reservoir in Khon Kaen Province at least 49 species of gastropod mollusk have been found, but although two species of the Bithyniidae were found to be included within the 9 most common species (*Wattebledia crosseana* and *W. siamensis*), *B. s. goniomphalos* was not [33]. Tesana [29] reported 10 species for freshwater gastropods from Lam Ta Khong Reservoir in Nahkon Ratchasima Province in the northeast of Thailand. Of these *B. s. goniomphalos* was the third most common after *Clea helena* and *Melanoides tuberculata*. It occurred throughout the lake, was most commonly found in the cooler season and was least abundant during the hot season. In a detailed study of the freshwater snails found in Lam Pao Dam and its associated irrigation areas, Sri-aroon et al. [31] found that *B. s. goniomphalos* was by far the most common of the 15 species found (89%) at all 6 sites sampled. In collections from 8 Provinces in northeast Thailand, Tesana et al. [34] found 8 species of gastropod of which *B. s. goniomphalos* ($n = 281$) was the second most common after *Pila pesmei* ($n = 454$).

In a major study of the freshwater mollusk fauna of Thailand, Sri-aroon et al. [35] collected specimens from 75 sites in 11 Provinces in the north and northeast of the country from 1999 to 2004. Thirty-nine species of gastropod from 9 families were collected. As expected, *B. funiculata* was

Table 1
The recent distribution records for the three *Bithynia* taxa which act as first intermediate hosts for *O. viverrini*.

Reference	Date	Species/subspecies of snail			Province	Region
		B. funiculata	*B. s. siamensis*	*B. s. goniomphalos*		
[7]	–	✓			Chiang Mai, Mae Hong son, Tak, Lampun, Chiang Rai	Northern
			✓		Bangkok, Chiang Mai, Tak, Lampun	Central, western, Northern and South eastern
				✓	Mahasarakam, Udon Thani	North eastern
[37]	05.1975–04.1976		✓		Bangkok	Central
[49]	05.1975–04.1976		✓		Bangkok	Central
[25]	01.1980–03.1984			✓	Khon Kaen	North eastern
[14]	–	✓			Chiang Mai	Northern
			✓		Bangkok	Central
				✓	Kalasin	North eastern
[15]	–	✓			Chiang Mai	Northern
			✓		Bangkok	Central
				✓	Kalasin	North eastern
[50]	–			✓	Khon Kaen, Sakon Nakhon	North eastern
[24]	1989–1990			✓	Khon Kaen	North eastern
[29]	1994			✓	Nakhon Ratchasima	North eastern
[29]	04.2000			✓	Nakhon Ratchasima	North eastern
[31]	12.2003			✓	Kalasin, Udon Thani	North eastern
[51]		✓			Chiang Mai	Northern
			✓		Bangkok	Central
				✓	Khon Kaen, Kalasin, Chaiyaphum, Roi Et, Nakhon Ratchasima, Burirum, Si Sa Ket, Ubon Ratchathani	North eastern
[41]	18.12.2003			✓	Kalasin	North eastern
[30]	06.2004–10.2004	✓			Chiang Mai	Northern
[35]	1999–2004	✓			Chiang Rai	Northern
			✓		Sukhothai, Phitsanulok	Central
				✓	Nong Khai, Khon Kaen, Kalasin, Nakhon Ratchasima, Ubon Ratchathani, Sakon Nakhon	North eastern
[52]	–	✓			Chiang Mai	Northern
			✓		Bangkok	Central
				✓	Khon Kaen	North eastern

found only in the far north, *B. s. siamensis* in the mid-north and *B. s. goniomphalos* in the northeast. Only 9 specimens of *B. funiculata* were found, all from a single district, comprising only 2.8% of the snails found there. Low abundance of *B. s. siamensis* was observed in the three districts in which it was found (4.4%, 9.7% and 38.3%, respectively). For *B. s. siamensis* the maximum number found at a sample site was 121 snails. *Bithynia s. goniomphalos*, however, comprised over 50% of snails collected in 6 of the 8 districts in which it was found (9.7%, 11.5%, 51.9%, 60.1%, 62.6%, 65.7%, 72.2%, 95.0%) with over 500 specimens being found at 4 of the 8 sites. The substantial variability in the relative abundance of *B. s. goniomphalos* in different geographical areas is associated with an almost complete lack of knowledge of those factors influencing the population dynamics of this species.

A study in a series of 11 natural ponds in Ayutthaya Province found 7 gastropod species of which *Melanoides tuberculata* (83.7%) dominated. *Bithynia s. siamensis* accounted for only 0.4% of the snails found and had a density of between 0.37 and 0.61 snails/m^2 [36]. It was highly overdispersed between ponds (variance to mean ratio 25.3).

6. Population dynamics

There are few studies of population parameters and dynamics in the *Bithynia* intermediate hosts of *O. viverrini*. An early study by Upatham and Sukhapanth [37] on *B. s. siamensis* from 5 standing water sites, carried out from May 1975 to April 1976, found that snail populations fluctuated with rainfall and that behavior was dependent on water level. In times of high rainfall when water levels were also high, more snails were recovered from the water surface than from the muddy bottom. During dry periods, snails were found on the surface as well as in and on the mud but with a higher percentage of individuals in the mud. In general, little or no population dynamic information is available for either *B. funiculata* or *B. s. siamensis*.

The most detailed study to date was carried out by Brockelman et al. [25] on a natural population of *B. s. goniomphalos* in Khon Kaen Province, Thailand. The study was conducted from September 1980 to March 1984 in a shallow, natural reservoir of about 15–20 ha. Snails were found both on the mud bottom at the center of the reservoir as well as in dense aquatic vegetation, confirming the results of Upatham and Sukhapanth [37]. Core and scoop samples were taken each month along transects reaching from the shore to 60 m into the lake and included mud cores as well as vegetation. Sampling by hand was biased towards larger individuals. Sampling began at the end of the rainy season in 1980. Little rain fell thereafter and the pond had dried out entirely by April 1982 and remained so until flooding took place in September 1982. Abundance was high at the start of the study, ranging between about 400 to 500 snails/m^2. With the onset of the drought this was markedly reduced to 5–10 snails/m^2. Snail mortality by the time the lake had dried out was slightly greater towards the center (99.8%) than at the edge (93%).

Lohachit [24] found variable dynamics dependent on the area in which *B. s. goniomphalos* were found. In rice fields, peak densities were observed in October and January (Bung Chim, 2 annual rice crops), November–December (Na Lom, 1 annual rice crops), July and February (resettlement village, 1 annual rice crops) and July–August and March-April (Non Swan, 1 annual rice crops). This enormous variability strongly suggests that the factors that regulate the populations of this species are not directly seasonally related.

Brockelman et al. [25] found rapid reproduction under suitable conditions, e.g. after spring rains and monsoon flooding. They cite laboratory studies which show that *B. s. goniomphalos* lay ca. 20 eggs/week and that the young reach maturity at 4–6 months (7 mm in length). The population doubling time in these laboratory experiments was 7 weeks (intrinsic rate of natural increase r = 0.10/week), but Brockelman et al. [25] consider that this was probably an underestimate for field populations. Core samples showed high levels of reproduction from December to March when water levels were high. Reproduction also increased two months after flooding in 1982. Brockelman et al. [25] suggest that when rainfall is adequate 2 generations can occur per year but they do not know whether this is also the case for larger bodies of permanent water.

The techniques used here must be carefully applied as all species appear to select different habitats dependent on the amount of water present [25,37]. When high water levels prevail, the snails attach to vegetation and dispersal is favored, while at low water levels the snails burrow into the mud to avoid desiccation. Brockelman et al. [25] overcame this problem by sampling into the sediment with a grab to a depth of 6 cm. Nevertheless, no profile studies have been made to determine the proportions of snails at different depths in relation to water level or how deeply they bury themselves in mud, particularly during drought periods.

Table 2
Prevalences of infection of *Bithynia* with *O. viverrini* cercariae.

Species	Country	Date	Prevalence	Reference
B. s. siamensis	Thailand	05.1975–04.1976	Mean 1.6%	[37]
B. s. goniomphalos	Lao PDR		Max. 2.2%	[40]
B. s. goniomphalos	Lao PDR	May to September	Mean 1.6%	[26]
S. s. siamensis	Lao PDR	May to September	0%	[26]
B. s. goniomphalos	Thailand	01.1980–03.1984	Mean 0.11% Max. 0.63%	[25]
B. funiculata	Thailand	06.2004–10.2004	Mean 0.28%	[30]
B. s. goniomphalos	Thailand	1994	0%	[29]
B. s. goniomphalos	Thailand	12.2003	0–1.3%	[31]
B. s. goniomphalos	Thailand		0.03–0.36%	[24]
B. s. goniomphalos	Thailand	01.2009–01.2010	Mean 2.95% (0.22–6.93%]	Kiatsopit et al. (unpublished data)
B. s. goniomphalos	Lao PDR	01.2009–01.2010	Mean 2.44% (0.37–8.37%]	Kiatsopit et al. (unpublished data)

7. Infection

Bithynia species in Thailand are infected with cercariae of at least 10 non-opistorchoid trematode species, all of which are quite different from the pleurolophocercous cercariae of *O. viverrini*[38]. Some intestinal trematodes also produce pleurolophocercous cercariae which could perhaps lead to misidentifications. Differentiation between the different species producing pleurolophocercous cercariae may not be easy [39]. The method we use to identify the *O. viverrini* cercaria was a combination of morphology (used by previous authors and found to be accurate for this species) and PCR to check species specific gene sequences. We also, when possible, used cercariae to infect fish and obtain metacerariae which were then given to hamsters to obtain adult worms for morphological confirmation.

Infection rates in all species of snails were low (0.003–2.4%) [37,40] until Kiatsopit et al. (unpublished data) recently discovered several populations in both Thailand and Laos with prevalences well above this, ranging up to 8.37% (Table 2). None of the 527 *B. s. goniomphalos* from the main freshwater reservoir in the Huai Tralaeng district, Nakhon Ratchasima Province in northeast Thailand were found to be infected, although this does not rule out very low prevalences [41]. The same applies to Lam Ta Khong Reservoir, Nakhon Ratchasima [29].

Giboda et al. [26] indicated that there appeared to be seasonal variation in the prevalence of infection of *B. s. goniomphalos* in paddy fields in Lao PDR, with all samples between May and June, usually the beginning of the rainy season, being negative. Samples from the nearby Nam Ngum reservoir showed no such seasonal effect. Unfortunately, Giboda et al. [26] did not provide their collection data showing sample sizes for each collection site and collection date, making it impossible to determine the likelihood of finding a positive snail given the very low overall prevalence (0.6%).

Brockelman et al. [25] observed changes in the prevalence of infection of *B. s. goniomphalos* with *O. viverrini* over a 45 month period between 1980 and 1984. This is the only study of its type; however the dramatic population crash in the snails which was associated with the prolonged drought beginning early in 1981 make it difficult to obtain accurate prevalence estimates. Samples taken from March 1981 to October 1982 contain too few snails. Clearly, if snail density is low and prevalence generally is low, it is easy to miss finding infected snails.

Brockelman et al. [25] found that only snails >8 mm were infected, i.e. those of reproductive size. However, Chanawong and Waikagul [42] found that immature laboratory bred *B. s. siamensis* of 2–4 mm were more susceptible to infection than mature field caught specimens (6–8 mm). Upatham and Sukhapanth [37] also found specimens of *B. s. siamensis* less than 4 mm diameter to be infected, but that the number of cercariae shed increased with shell size.

Studies of the micro-scale pattern of snail infection are completely unknown, although this is likely to be dictated by hydrological flow patterns bringing eggs from infected fecal material into the snail's habitat. Such flow patterns should be of considerable epidemiological significance and research in this area could potentially be of considerable value.

Only a single study on host mortality in relation to intensity of infection has been carried out to date [42]. *Bithynia s. siamensis* snails were fed 30, 50 or 90 eggs over a two day period. The more *O. viverrini* eggs ingested, the greater the likelihood of infection (Table 3). Snail mortality also increased in a linear fashion with intensity of infection from 10% with 30 eggs ingested to 30% with 90 eggs ingested. This was significantly higher than mortality in uninfected snails indicating that *O. viverrini* infection is pathogenic for the snail hosts.

A reanalysis of the data presented by Chanawong and Waikagul [42] showed that both laboratory-reared and field caught *B. funiculata* and *B. s. siamensis* showed higher infection rates than *B. s. goniomphalos*. Mortality was significantly higher in field-caught snails than laboratory specimens for *B. funiculata* ($\chi^2_1 = 16.1$, $P < 0.001$) and *B. s. siamensis* ($\chi^2_1 = 15.6$, $P < 0.001$), but not for *B. s. goniomphalos* (Fisher's exact test, $P = 0.12$), although few specimens were available for the latter species (Table 3). Laboratory-reared *B. funiculata* ($\chi^2_1 = 8.03$, $P < 0.005$) and *B. s. siamensis* ($\chi^2_1 = 13.4$, $P < 0.001$) showed higher infection rates than field caught snails, while *B. s. goniomphalos* did not ($\chi^2_1 = 0.06$, n.s.). This indicates that accurate epidemiological parameters cannot be obtained from laboratory snail colonies, and suggests that *B. s. goniomphalos* may respond differently to infection than the other two taxa.

Contradictory evidence from a single study has been provided by Chanawong and Waikagul [42] but it is not possible to determine whether co-evolution between populations has occurred.

Phongsasakulchoti et al. [43] observed the cercarial output from 5 *B. s. goniomphalos* collected from Kalasin Province, northeast Thailand. The total number of cercariae shed ranged from 2,136 to 27,692 with a mean of 11,311. The mean shedding duration was 36 days (range 22–61 days). Shedding followed a circadian pattern peaking in mid-morning around 09.00 and dropping off steeply after midday. No shedding occurred at night.

8. Control

Haruey et al. [32] suggested a potential food competitor for *B. s. goniomphalos*, *Filopaludina m. martensi* could be used as a biological control agent to reduce *Bithynia* numbers. Such introductions have proven successful on a number of occasions, particularly against *Schistosoma mansoni* in the Caribbean area where the introduction of a non-susceptible competitor gastropod, *Melanoides tuberculata*, led to interruption of the transmission cycle and the near-total disappearance

Table 3
Experimental infection of laboratory-bred and filed-collected *Bithynia* with *O. viverrini* cercariae. Data from Chanawong and Waikagul [42].

Species	Group	Infected snails		Not infected
		Alive	Dead	
B. funiculata	Laboratory	51	32	32
	Field	2	17	21
B. s. goniomphalos	Laboratory	8	8	151
	Field	0	4	36
B. s. siamensis	Laboratory	65	44	47
	Field	2	17	28

of the native intermediate host [44]. However, more detailed information and considerable research is needed before such a procedure could be recommended for *B. s. goniomphalos*.

Thammasiri et al. [28] have shown that the molluscicide Bayluscide (niclosamide] is effective against *B. s. goniomphalos* in the laboratory, but have pointed out that the behavior of the snail, which can live in deep water and in mud during the dry season, may prevent effective contact between the agent and its victim.

It is of potential significance that of the three taxa of *Bithynia* which can act as first intermediate hosts of *O. viverrini*, *B. s. goniomphalos* is by far the most frequently found, being the dominant gastropod species in parts of northeast Thailand. These characteristics together with rapid growth and high reproductive capabilities under suitable conditions make it potentially a successful invading species [45]. A similar situation is found with the highly successful invaders *Bithynia tentaculata* which is of Eurasian origin but which has colonized the northeast USA and Canada, and Upper Mississippi River where it is also a vector of introduced waterfowl parasites [46–48]. Should *B. s. goniomphalos* (or one of the other first intermediate hosts) be introduced elsewhere, the possibility of the introduction of *O. viverrini* should be seriously considered.

9. Conclusions

Our knowledge of the ecology of the *Bithynia* hosts of *O. viverrini* is rudimentary and fragmented. The older publications could not take into account the restriction of populations or even species to different wetlands systems and generalizations about the currently recognized taxa or population genetic groups is not possible without comparing information between such wetlands. Even this may be inadequate as substantial variation in prevalence and incidence of infection in the second intermediate fish hosts, the ecology of which is poorly known, have been found between areas as little as 40 km apart, indicating that local factors may override general processes (Sithithaworn et al. unpublished data). Indeed, this review only deals with one phase in the life cycle of *O. viverrini*; the phase involving the large number of cyprinid second intermediate hosts remains to be explored.

Detailed, long-term studies within different wetland systems are urgently needed to clarify both distributional parameters as well as the interpopulation variation in population dynamics. Given the rapid rate of change in the Mekong Subregion based on such factors as human population density, pollution, land-use change, modification of hydrological patterns and climate change, it is urgent that these studies be carried out as soon as possible to provide a baseline with which future studies can be compared.

Although this review only considers the *Bithynia* species and subspecies involved in the transmission cycle of *O. viverrini*, it is important to remember that cyprinid fish are also essential for human infection. Ecological information on these second intermediate hosts is also rare, and the situation is much more complex than that of the snails due to the wide variety of fish species involved. These species also need to be incorporated into comprehensive study programs.

Acknowledgements

We sincerely thank the Thai Research Foundation-Royal Golden Jubilee for funding Dr. T. Petney's trip to Thailand to discuss the ecological processes involved in the *O. viverrini* life cycle and the support of the Basic Research Grant, Thailand Research Fund. This work was also supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through health cluster (SHeP-GMS), Khon Kaen University. We also thank the Faculty of Medicine Visiting International Professor Program.

References

[1] Andrews RH, Sithithaworn P, Petney TN. *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol 2008;24:497–501.
[2] Haas W, Granzer M, Brockelman CR. *Opisthorchis viverrini*: finding and recognition of the fish host by the cercariae. Exp Parasitol 1990;71:422–31.
[3] Petney TN. Environmental, cultural and social changes and their influence on parasite infections. Int J Parasitol 2001;31:919–32.
[4] Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Pipitgool V, Tesana S, et al. Evidence of a species complex within the food-borne trematode *Opisthorchis viverrini* and possible co-evolution with their first intermediate hosts. Int J Parasitol 2007;37:695–703.
[5] Songtham W, Ugai H, Imsamut S, Marinate S, Tansathien W, Meesook A, et al. Middle Miocene molluscan assemblages in Mae Moh Basin, Lampang Province, Northern Thailand. Science Asia 2005;31:183–91.
[6] Boonchai N, Grote PJ, Jintasakul P. Paleontological parks and museums and prominent fossil sites in Thailand and their importance in the conservation of fossils. In: Lipps JH, Granier BRC, editors. PaleoParks — the protection and conservation of fossil sites worldwide. Brest: Notebooks on Geology; 2009. p. 75–95.
[7] Brandt RAM. The non-marine aquatic Mollusca of Thailand. Arch Molluskenkd 1974;105:1–423.
[8] Harinasuta C, Harinasuta T. *Opisthorchis viverrini*: life cycle, intermediate hosts, transmission to man and geographical distribution in Thailand. Arzneimittelforschung 1984;34:1164–7.
[9] Sithithaworn P, Yongvanit P, Tesana S, Pairojkul C. Liver Flukes. In: Murrell KD, Fried B, editors. Parasitic Foodborne Zoonoses. Springer Verlag; 2008. p. 1–51.
[10] Davis GM. Historical and ecological factors in the evolution, adaptive radiation, and biogeography of freshwater mollusks. Am Zool 1982;22:375–95.
[11] Strong EE, Gargominy O, Ponder WF, Bouchet P. Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. Hydrobiologia 2008;595:149–66.
[12] Chung PR. A comparative study of three species of Bithyniidae (Mollusca: Prosobranchia): *Parafossarulus manchouricus*, *Bithynia tentaculata* and *B. (Gabbia) misella*. Malacol Rev 1984;17:1–66.
[13] Chitramvong YP, Upatham ES. A new species of freshwater snail for Thailand. Walkerana 1989;3:179–86.
[14] Chitramvong YP. The Bithyniidae (Gastropoda: Prosobranchia) of Thailand: comparative internal anatomy. Walkerana 1991;5:161–206.
[15] Chitramvong YP. The Bithyniidae (Gastropoda: Prosobranchia] of Thailand: comparative external morphology. Malacol Rev 1992;25:21–38.
[16] Glöer P, Yildirim MZ. Some records of Bithyniidae from Turkey with the description of *Bithynia pesicii* n. sp. (Gastropoda: Bithyniidae). Malakol Abh 2006;24:37–42.
[17] Glöer P, Albrecht C, Wilke T. Enigmatic distribution patterns of the Bithyniidae in the Balkan Region (Gastropoda: Rissooidea). Mollusca 2007;25:13–22.
[18] Glöer P, Pesic V. The *Bithynia* species from Skadar Lake (Montenegro) (Gastropoda: Bithyniidae). Mollusca 2007;25:7–12.
[19] Ponder WF, Colgan DJ. What makes a narrow-range taxon? Insights from Australian freshwater snails. Invert Syst 2002;16:571–82.
[20] Kiatsopit N, Sithithaworn P, Boonmars T, Tesana S, Chanawong A, Saijuntha W, et al. Genetic markers for studies on the systematics and population genetics of snails, *Bithynia* spp., the first intermediate hosts of *Opisthorchis viverrini* in Thailand. Acta Trop 2011;118:136–41.
[21] Rintelen TV, Bouchet P, Glaubrecht M. Ancient lakes as hotspots of diversity: a morphological review of an endemic species flock of *Tylomelania* (Gastropoda: Cerithioidea: Pachychilidae) in the Malili lake system on Sulawesi, Indonesia. Hydrobiologia 2007;592:11–94.
[22] Prasankok P, Srikoom W, Panha S. Genetic variation amongst viviparid snails in the genus Mekongia (Prosobranchia: Viviparidae) in Thailand. Biochem Syst Ecol 2009;37:452–8.
[23] Hoagland KE, Davis GM. The stenothyrid radiation of the Mekong River 1. The *Stenothyra mcmulleni* complex (Gastropoda: Prosobranchia). Proc Acad Nat Sci Philadelphia 1979;131:191–230.
[24] Lohachit C. Ecological studies of *Bithynia siamensis goniomphalos*, a snail intermediate host of *Opisthorchis viverrini*, in Khon Kaen Province, Northeast Thailand. Malacol Rev 2004–2005;37/38:1–26.
[25] 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.

[26] Giboda M, Ditrich O, Scholz T, Viengsay T, Bouaphanh S. Human *Opisthorchis* and *Haplorchis* infections in Laos. Trans R Soc Trop Med Hyg 1991;85:538–40.
[27] 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.
[28] Thammasiri C, Tesana S, Suwannatrai A, Pirate S, Khampoosa P, Kulsantiwong J, et al. Molluscicidal effects of Bayluscide (niclosamide) on *Bithynia siamensis goniomphalos*, first intermediate host of liver fluke, *Opisthorchis viverrini*. KKU Res J 2009;9:107–17.
[29] Tesana S. Diversity of mollusks in the Lam Ta Khong reservoir, Nakhon Ratchasima, Thailand. Southeast Asian J Trop Med Public Health 2002;33:733–8.
[30] Ngern-klun R, Sukontason KL, Tesana S, Sripakdee D, Irvine KN, 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.
[31] 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.
[32] Haruay S, Tesana S, Suwannatrai A, Piratae S, Khampoosa P, Thammasiri C, et al. Effects of surface salt on distribution and density of snails in Family Viviparidae in Khon Kaen province, Thailand, analyses by using geographic information system. KKU Res J 2008;8:40–50.
[33] Kittivorachate R, Yangyuen C. Molluscs in the Ubolratana Reservoir, Khon Kaen. Kasetsart J (Nat Sci) 2004;38:131–9.
[34] Tesana S, Srisawangwong T, Sithithaworn P, Laha T, Andrews R. Prevalence and intensity of infection with third stage larvae of *Angiostrongylus cantonensis* in mollusks from Northeast Thailand. Am J Trop Med Hyg 2009;80:983–7.
[35] Sri-aroon P, Butraporn P, Limsoomboon J, Kaewpoolsri M, Chusongsang Y, Charoenjai P, 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.
[36] Poomsripanon J. The enhancement of an understanding the nature of science by the use of GIS for study the mollusc ecology through 5Es learning cycle model. [PhD thesis]. Bangkok: Mahidol University; 2006.
[37] 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.
[38] Stock B. The diversity of trematode cercarial infections in *Bithynia* snails in northeast Thailand. [MSc thesis]. Karlsruhe Institute of Technology: Karlsruhe; 2011.
[39] Chuboon S, Wongsawad C. Molecular identification of larval trematode in intermediate hosts from Chiang Mai, Thailand. Southeast Asian J Trop Med Hyg 2009;40:1216–20.
[40] Ditrich O, Scholz T, Giboda M. Occurrence of some medically important flukes (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir, Laos. Southeast Asian J Trop Med Public Health 1990;21:482–8.
[41] 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(Suppl 3):106–9.
[42] 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.
[43] Phongsasakulchoti P, Sri-aroon P, Kerdpuech Y. Emergence of *Opisthorchis viverrini* cercariae from naturally infected *Bithynia* (*Digoniostoma*) *siamensis goniomphalos*. Southeast Asian J Trop Med Public Health 2005;36(Suppl 4):189–91.
[44] Petney TN, Taraschewski H. Water-borne parasitic diseases: hydrology, regional development and control. Water Chemistry and Microbiology. In: Frimmel FH, editor. A Treatise on Water ScienceAmsterdam: Elsevier; 2011.
[45] McMahon RF. Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Can J Fish Aquat Sci 2002;59:1235–44.
[46] Lipitzki DAW. Epizootiology and transmission of snail-inhabiting metacercariae of the duck digeneans *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus*. [PhD thesis]. Montreal: McGill University; 1993.
[47] Mitchell AJ, Cole RA. Survival of the faucet snail after chemical disinfection, pH extremes, and heated water bath treatments. N Am J Fish Manag 2008;28: 1597–600.
[48] Henningsen JP, Lance SL, Jones KL, Hagen C, Laurila J, Cole RA, et al. Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae). Conserv Genet Resour 2010;2:247–50.
[49] Upatham ES, Sukhapanth N. Field studies on the bionomics of freshwater snails of medical importance in Bangna, Bangkok, Thailand. Southeast Asian J Trop Med Public Health 1981;12:204–8.
[50] Adam R, Arnold H, Pipitgool V, Sithithaworn P, Hinz E, Storch V. Studies on lophocercous cercariae from *Bithynia siamensis goniomphalos* (Prosobranchia: Bithyniidae). Southeast Asian J Trop Med Public Health 1993;24:697–700.
[51] Kodcharin P. Genetic variation in *Bithynia siamensis goniomphalos*, first intermediate host of *Opisthorchis viverrini* in the basin of Mun and Chi rivers, Thailand by RAPD. [MSc thesis]. Khon Kaen: Khon Kaen University; 2005.
[52] Duangprompo W. Genetic variation in snails of the family Bithyniidae in Thailand and identification of *Bithynia siamensis goniomphalos* by PCR. [MSc Thesis]. Khon Kaen: Khon Kaen University; 2007.