Surveillance on Fish-Borne Parasites in Second Intermediate Hosts from Mekong Region of Myanmar, North and Northeast of Thailand

Ei Ei Phyo Myint

A Thesis Submitted in Partial Fulfillment of Requirements for
degree of Doctor of Philosophy in Health Sciences (International Program)
March 2021

การเฝ้าระวังของพยาธิที่ติดต่อจากปลาในโฮสต์กึ่งกลางตัวที่สอง บริเวณแม่น้ำโขงของประเทศพม่ากับภาคเหนือและภาคตะวันออกเฉียงเหนือ ประเทศไทย

วิทยานิพนธ์

ของ

Ei Ei Phyo Myint

เสนอต่อมหาวิทยาลัยมหาสารคาม เพื่อเป็นส่วนหนึ่งของการศึกษาตามหลักสูตร

ปริญญาปรัชญาดุษฎีบัณฑิต สาขาวิชาวิทยาศาสตร์สุขภาพ (หลักสูตรนานาชาติ)

มีนาคม 2564

Surveillance on Fish-Borne Parasites in Second Intermediate Hosts from Mekong Region of Myanmar, North and Northeast of Thailand

Ei Ei Phyo Myint

A Thesis Submitted in Partial Fulfillment of Requirements

for Doctor of Philosophy (Health Sciences (International Program))

March 2021

The examining committee has unanimously approved this Thesis, submitted by Ms. Ei Ei Phyo Myint , as a partial fulfillment of the requirements for the Doctor of Philosophy Health Sciences (International Program) at Mahasarakham University

Examining Committee

..... Chairman
(Chairat Uthaipibull , Ph.D.)

..... Advisor
(Asst. Prof. Choosak Nithikathkul , Ph.D.)

..... Co-advisor
(Asst. Prof. Amornpun Sereemaspun , Ph.D.)

..... Committee
(Assoc. Prof. Pramote Thongkrajai , Ph.D.)

..... Committee
(Asst. Prof. Darunee Puangpronpitag , Ph.D.)

..... External Committee
(Sauwanan Bumrerraj , Ph.D.)

Mahasarakham University has granted approval to accept this Thesis as a partial fulfillment of the requirements for the Doctor of Philosophy Health Sciences (International Program)

....

(Asst. Prof. Teabpaluck Sirithanawuthichai , Ph.D.)
Dean of The Faculty of Medicine

(Assoc. Prof. Krit Chaimoon , Ph.D.)
Dean of Graduate School

TITLE Surveillance on Fish-Borne Parasites in Second Intermediate Hosts from Mekong Region of Myanmar, North and Northeast of Thailand

AUTHOR Ei Ei Phyo Myint

ADVISORS Assistant Professor Choosak Nithikathkul , Ph.D.
Assistant Professor Amornpun Sereemaspun , Ph.D.

DEGREE Doctor of Philosophy **MAJOR** Health Sciences (International Program)

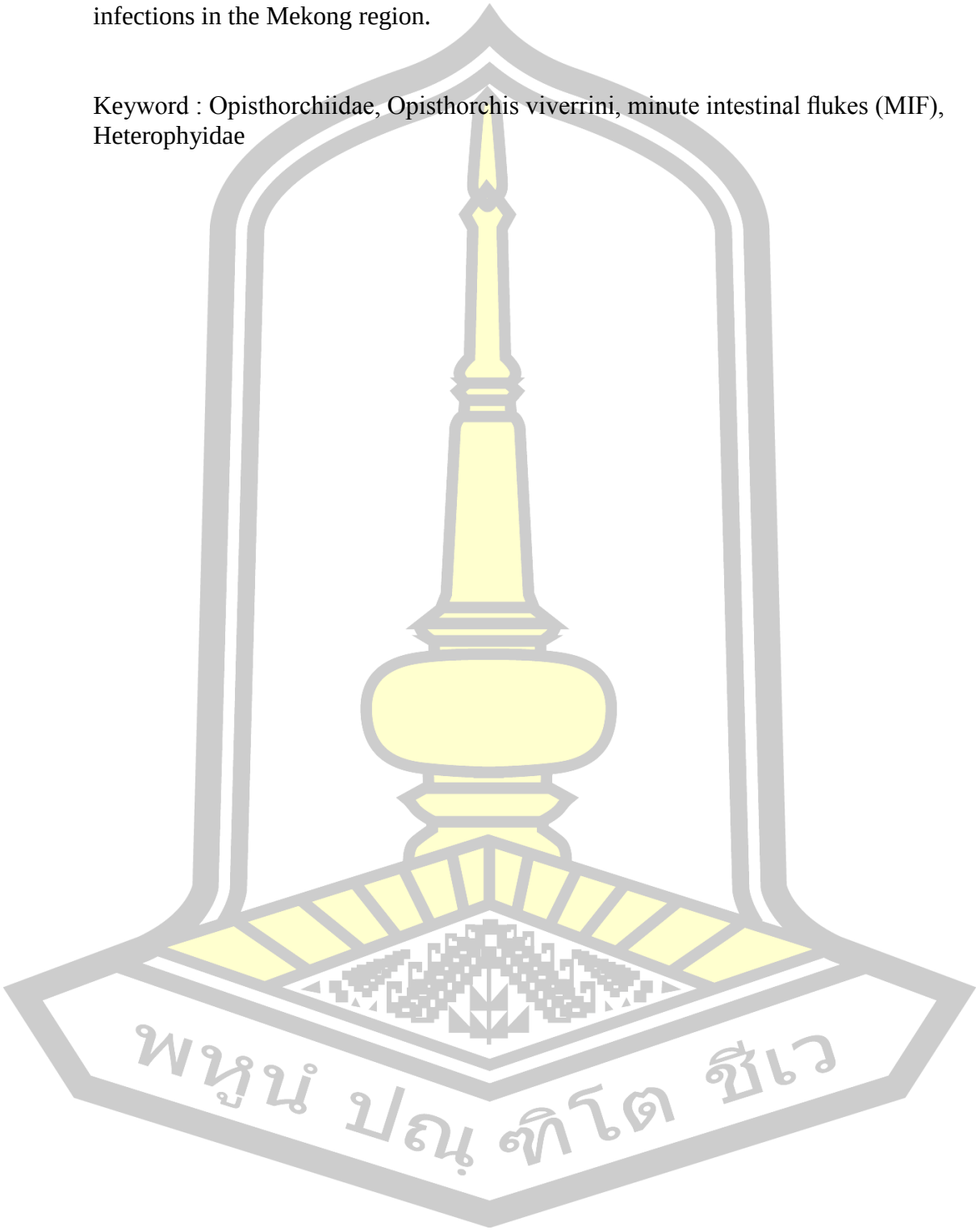
UNIVERSITY Mahasarakham University **YEAR** 2021

ABSTRACT

Countries of lower Mekong regions are highly alarmed by the spread of fish borne trematode infections i.e. small liver flukes and minute intestinal flukes especially in Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia and Myanmar. Moreover, the incidence of cholangiocarcinoma, a major primary carcinoma of the liver with a very poor prognosis has also been increasing in the endemic area of liver fluke infections. In Thailand, the epicenter of this disease is located in north and northeast region, where high a prevalence of opisthorchiasis coexists with a high incidence of cholangiocarcinoma. Only a few reports have been published concerning the fish borne trematodes infections in the central region of Myanmar. However, there is still a lack of information regarding the status of trematodes infections in second intermediate hosts in the Mekong region of Myanmar. A total of 2884 fishes (48 species) were investigated from total 8 locations from Mekong region of Myanmar, central region of Myanmar, north of Thailand and northeast of Thailand ie; Tachileik, Bago, Chom Thong, Mae Ngat, NikhomKham Soi, Sakon Nakhon, Sisaket, Kalasin. The collected fishes were examined for presence of the infective stage of the parasite, i.e., metacercariae by pepsin-HCl artificial digestion techniques. Detected metacercariae were observed and identified using a stereomicroscope and light microscope. A GIS database for the study of fish borne trematode metacercariae was implemented using Arc Map 10.5 software. A total of 1359 fishes (27 species) were positive with fish borne trematode matacercariae infections. Four Species of fish borne trematode metacercariae i.e.; small liver fluke, *Opisthorchis viverrini*, minute intestinal flukes *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchoides sp*. were detected in this study. The geographic information (latitude and longitude) associated with the infection rates among susceptible species of fresh water fish was recorded and built a fish borne geo-dataset for Geographical Information System (GIS) development. GIS can be useful in establishing strategies for the prevention of transmission of food borne diseases originating in infected fish found in water catchment areas. The outcome of this study could be a useful index for the fish borne zoonotic trematode epidemiology in the

Mekong area. Besides, the results of our study contribute to filling the gap of information necessary for the control and prevention of fish borne trematode zoonotic infections in the Mekong region.

Keyword : Opisthorchiidae, Opisthorchis viverrini, minute intestinal flukes (MIF), Heterophyidae



ACKNOWLEDGEMENTS

I would like to express my deepest and sincere gratitude to my advisor, Assistant Professor Dr Choosak Nithikethkul for his kind supervision and valuable guidance during this thesis work. I also would like to express my special gratitude to my co advisor Assistant Professor Amornpun Sereemaspunb for his valuable supervisions and recommendations.

I am greatly appreciated the financial support received through a research grant from The joint TICA–TRF–Sida–ISP scholarship for doctoral studies, co-sponsored by the Thailand International Cooperation Agency (TICA), the Thailand Research Fund (TRF) (under the Royal Golden Jubilee Ph.D. Program), and the Swedish International Development Cooperation Agency (Sida).

I am gratefully thanked to teaching staffs and all the faculty members from faculty of Medicine, Mahasarakhum University, Thailand.

I would like to extend my most heartfelt thanks to research affairs division staffs for their moral encouragement and kind support for this thesis work.

Finally, I would like to express my sincere gratitude and appreciation to my dear parents who give me a chance to study and have strongly supported me.

Ei Ei Phyo Myint

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

1.1 Backgrounds

Zoonotic trematode (ZT) infections are an important public health problem in many Asian countries, including Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia, Thailand, the Philippines, China, Taiwan, and the Republic of Korea (Korea) (Sanpool et al., 2018) (Sohn, 2009) (Do et al., 2007) (Rim et al., 2008). Especially, fish borne trematodes (FBT) provoke a remarkable morbidity in residents of these countries and cause a serious economic damage in the industry of fish aquaculture (Chai et al., 2005) (Chai et al.,2007) Currently, more than 45 million people estimated to be infected (Hung et al., 2013). Small liver flukes, Opisthorchiidae and minute intestinal flukes (MIF), Heterophyidae are highly prevalent in Southeast Asian countries. Embryonated eggs containing miracidium are discharged in the biliary ducts and passed in the stool. Eggs are ingested by a suitable snail intermediate host (First intermediate host). Within the snail, eggs hatch to release miracidia, which transform to sporocysts (Anh et al., 2009). Then sporocysts undergo asexual reproduction to give rise to rediae and finally cercariae. Cercariae are released from the snail and after a short period of free-swimming time in water, it penetrates the skin of freshwater fish (second intermediate host), where it encysts as a metacercaria (Dao et al., 2017). Humans are infected through ingestion of undercooked or raw freshwater fishes. The metacercariae excyst in the duodenum and ascend the biliary tract through the ampulla of Vater. Maturation to adult worms of trematode infection inhabit the intra and extrahepatic biliary system (Pumidonming et al., 2018) The human liver flukes, *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis* remain important public health problems in many parts of the world, particularly in Asia (Keiser et al., 2009) (Dorny et al., 2009) *Clonorchis sinensis* is endemic in southern China, Korea and northern Vietnam, whereas *O. viverrini* is endemic in the Lower Mekong Basin, including Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia and central Vietnam and Myanmar

(Sithithaworn et al., 2012) (Pyo et al., 2013) *Opisthorchis felineus* is found in Central–Eastern Europe (Lansing et al 2019). It has been known that more than 50 million people are infected with intestinal trematodes, and about 70 trematode species are involved in human infections around the world (Dorny et al., 2009) (Prakobwong et al., 2017) (Suwannatrai et al., 2018)

Particularly, they are prevalent in Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia, Thailand, Myanmar, the Philippines, China, Taiwan, and the Republic of Korea (Radomyos et al., 1998) (Chai et al., 2005) (Chai et al., 2009) (Dung et al., 2007) (Phan et al., 2010). Countries of lower Mekong regions are highly alarmed the spread of fish borne trematode infections i.e. small liver flukes and minute intestinal flukes especially in Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia and Myanmar. (Do et al., 2007) (Saenphet et al., 2008) (Lovis et al., 2009). Fish-borne zoonotic trematodes (FZT) especially small liver flukes (Opisthorchiidae) and minute intestinal flukes (Heterophyidae) are highly prevalent in that regions (Jeon et al., 2012). These two flukes have the similar life cycles and involved two intermediate hosts to complete their life cycle (Boerlage et al 2013) (Anh et al., 2009). The first intermediate hosts are snails and the second intermediate hosts are small freshwater fishes (Dao et al., 2017). In southeastern Asia, more than 90 million people are at risk of infection, and at least 10 million people are estimated to be infected by *O. viverrini* .More than ten million people are estimated to be infected with *O. viverrini*: about eight million in Thailand and two million in Lao People's Democratic Republic (Sripa et al., 2011). The highest prevalence occurs in North and Northeast Thailand, especially in rural populations (Sripa et al., 2011) (Jongsuksuntigul et al., 2003) (Yamagishi et al., 2011) and in the adjacent southern and central regions of Lao People's Democratic Republic (Forrer et al., 2012; Sayasone et al., 2009). Human infections with *O. viverrini* and presence of metacercariae in intermediate hosts have been reported in several provinces of Cambodia (Chai et al., 2014) (Miyamoto et al., 2014) (Sohn et al., 2012) (Yong et al., 2014). Miyamoto et al reported *O. viverrini* eggs in human fecal samples from 26 out of 55 surveyed villages in five provinces of Cambodia, among which 15 villages had an egg positive rate >10% (Miyamoto et al., CM, 2014). The parasite is also endemic in southern and central parts of Vietnam (Dao et al., 2016) (Dung et al., 2014). A

survey conducted in 2015 reported that the overall prevalence of *O. viverrini* infection was 11.4% in central Vietnam (Dao et al., 2016). The main cause of infection is due to the habit of eating raw or partially cooked meat or fish. When a human consumes raw or semi-cooked fish that may contain metacercariae of the liver fluke and then adult worms inhabit at the bile ducts, where they feed on epithelial cells (Sripa et al., 2010). Most complications of chronic human opisthorchiasis are hepatobiliary diseases such as hepatomegaly, cholangitis, cholecystitis, peri-ductal fibrosis and gallstones. Recently, an association with cholangiocarcinoma has also been demonstrated (Honjo et al., 2005) (Ayé et al., 2015).

Humans are being infected through ingestion of undercooked or raw freshwater fishes which is contaminated with infective stage of parasite i.e. metacercariae. (Pumidonming et al., 2018). Traditional habit of eating raw or undercooked fish is a known risk factor for human trematode infections. (Sithithaworn et al., 2012) (Paiboon et al 2012). Moreover, Infection can also occur via poor personal hygiene and the contamination of food, hands and food preparation utensils that are contaminated with metacercariae. In Myanmar, we also have the traditional habits of eating raw small cyprinoid fishes pickled with rice, locally called (Nyar lay Chin) (Figure).

Figure 1 Photos of local Myanmar food

(a) Raw small cyprinoid fishes pickled with rice (Nyar lay Chin)

(b) Nyar lay Chin prepared as uncooked salad mixed with onion (Nyar lay Chin salad).

Opisthorchiasis caused by carcinogenic liver fluke, *O. viverrini* has been remained important public health problems in lower Mekong region countries i e Cambodia, Lao People's Democratic Republic, Vietnam, and Thailand), where there is a culture of eating raw or undercooked fish that contain encysted metacercariae of the liver fluke (Hung et al., 2013) (Sripa et al., 2011) (Sripa et al., 2008). Adult flukes are long-lived and feed on epithelial cells of the intrahepatic bile ducts. Although most cases are asymptomatic, complications can include hepatobiliary diseases such as hepatomegaly, cholangitis, cholecystitis, peri-ductal fibrosis and gallstones. Severe chronic infection is a strong risk factor for cholangiocarcinoma (Honjo et al., 2005) (Zheng et al., 2017). The incidence of cholangiocarcinoma has also been increasing in the endemic area of liver fluke infections.

In Myanmar, there is one report in 2019 about a retrospective study of the cholangiocarcinoma cases admitted to the Hepatobiliary and Pancreatic surgery department, Yangon Specialty Hospital, Myanmar, the prevalence has notably been increasing since 2016 and the highest prevalence is in 2018 (Kyaw et al., 2019). For the increasing data of cholangiocarcinoma case from Myanmar, the associated risk factor such as carcinogenic liver fluke infections and epidemiological data need to be more surveillance for the control and prevention program. Very recently, human infection with *O. viverrini* has been reported from rural people in Myanmar in the lower Irrawaddy and Salween basins (Aung et al., 2017). Identification of the parasite in Myanmar was based on fecal examination and molecular identification of eggs (Aung et al., 2017). In surveyed communities living in the Irrawaddy Delta, prevalence in the Bago Region 18.9% and in the Yangon Region it was 3.6%. In the Mon State, around the lower Salween River, prevalence was 5% (Aung et al., 2017). In 2017, a molecular study detected a mitochondrial cytochrome c oxidase subunit I (cox1) gene of *O. viverrini* from the fecal samples of persons in a rural area near Yangon (Aung et al., 2017). however, adult flukes were not recovered from the egg-positive persons. Another study in 2018 detected *O. viverrini* metacercariae from freshwater fish (*Puntius brevis*) caught in central Myanmar and obtained adult flukes from experimentally infected hamsters (Sanpool et al., 2018). Heterophyidiasis is an infection of the small bowel by minute intestinal flukes of the genus Heterophyes or related members of the family Heterophyidae (Krailas et al., 2016). More than 21

species of heterophyid flukes have been reported in humans (Waikagul et al 1991). Seven species of minute intestinal flukes (members of the Heterophyidae) have been reported in Yangon, Myanmar. However, there is still lack of information about the fish borne trematode infection status from Mekong region of Myanmar. 1.*Haplorchis taichui,* 2. *H. pumilio,* 3. *H. yokogawai*,4. *Centrocestus spp.,* 5. *Stellantchasmus falcatus, 6. Pygidiopsis cambodiensis*, and 7. *Procerovum sp.* were detected in Yangon Myanmar. There is a few report for trematode metacercariae infections in the second intermediate host among the regions of Myanmar except for Aung et al (Aung et al., 2017) which reported the presence of the liver fluke in faecal sample. In 2018, Sanpool et al., reported that finding of *O. viverrini* in cyprinoid fish in Central Myanmar. The Republic of the Union of Myanmar (Myanmar) is a sovereign state in the region of Southeast Asia, and bordered by India and Bangladesh to its west, Thailand and Lao PDR to its east, and China to its north and northeast. Administratively, it is divided into 7 states and 7 regions (formerly called divisions). Myanmar has similar agro-based ecology and environmental risks as others in the Greater Mekong sub-region leading to the broad array of fish borne trematode infections. Bago is located in the southern central part of the country. It is bordered by Magway Region and Mandalay Region to the north; Kayin State, Mon State and the Gulf of Martaban to the east; Yangon Region to the south and Ayeyarwady Region and Rakhine State to the west. It is located between 46°45'N and 19°20'N and 94°35'E and 97°10'E. It has a population of 4,867,373 (2014) (Callahan, 2017). Tachileik is a town which is situated in Mekong basin of Myanmar. Geographically, it is involved in the Golden Triangle that is the area where the borders of Thailand, Laos, and Myanmar meet at the confluence of the Ruak and Mekong rivers. Since there is no report concerning the distribution of metacercariae from Mekong region in Myanmar, the present investigation was undertaken to determine the intensity and distribution of trematode metacercariae in natural cyprinoid fish collected from Mekong region in Myanmar compare with north and north east of Thailand. Human infections with *O. viverrini* and presence of metacercariae in intermediate hosts have been reported in several provinces of Cambodia (Chai et al., 2014) (Miyamoto et al., 2014) (Sohn et al., 2012) (Yong et al., 2014). Miyamoto et al reported *O. viverrini* eggs in human fecal samples from 26 out of 55 surveyed villages in five provinces of Cambodia,

among which 15 villages had an egg positive rate >10% (Miyamoto et al., 2014). The parasite is also endemic in southern and central parts of Vietnam (Dao et al., 2016) (Dung et al., 2014). A survey conducted in 2015 reported that the overall prevalence of *O. viverrini* infection was 11.4% in central Vietnam (Dao et al., 2016). Although not many reported to date from Myanmar, the presence of O. viverrini is likely because of its close proximity to endemic areas in Thailand and because of an open-borders policy that started in 2015 leading to increasing migration among ASEAN Economic Community (AEC) countries (Thailand, Lao People's Democratic Republic, Cambodia, Vietnam and Myanmar) (ASEAN Economic Community Blueprint. Declaration on the ASEAN Economic Community blueprint 2008. http://asean.org/wp-content/uploads/archive/51 87-10.pdf Accessed 4Mar 2017) .Approximately eight million people in Thailand and two million in Lao PDR are estimated to be infected with *O. viverrini* (Sripa et al., 2010) with high prevalences reported in rural populations of North and Northeast Thailand (Sripa et al., 2011; Jongsuksuntigul and Imsomboon,, 2003; Sithithaworn et al., 2012) and in the adjacent central and southern parts of Lao PDR (Forrer et al., 2012; Sayasone et al., 2009). In Thailand, the epicenter of Opisthorchis viverrini infection is located in north and northeast Thailand, where high a prevalence of opisthorchiasis coexists with a high incidence of cholangiocarcinoma (CHCA). Southeast Asian liver fluke (*Opisthorchis viverrini)* and Chinese liver fluke (*Clonorchis sinensis*) are classified as Group 1 carcinogens, i.e. they are substantiated and directly cancer-causing agents (Bouvard et al., 2009). *O. viverrini* is a food-borne liver fluke that mainly attacks the area of the bile duct. Infection with the parasite, called opisthorchiasis is the major cause of cholangiocarcinoma, a cancer of the bile ducts, in northern Thailand, the Lao People's Democratic Republic, Vietnam and Cambodia. (Do et al., 2007; 'Saenphet et al., 2008; Lovis et al., 2009; Sithithaworn et al., 2003; Chai et al., 2017; Wattanayingcharoenchai et al., 2011; Jeon et al., 2012). A GIS database for the study of fish-borne metacercariae implemented using an ArcGIS Desktop program from the ESRI Company, Bangkok, Thailand. A geographic information system (GIS) is a framework for gathering and analyzing data. GIS integrates many types of data. It analyzes spatial location and organizes layers of information into visualizations using maps and 3D scenes. With this unique capability, GIS reveals deeper insights into

data, by helping users make smarter decisions. In our study, we investigated environmental factors that influence to fish-borne metacercariae, the GIS database was overlaid with population density and precipitation GIS datasets. Geographic coordinates of each area are determined with a Global Positioning System. Maps are the geographic container for the data layers. GIS maps are easily shared and embedded in apps and accessible by virtually everyone, everywhere (William et al., 1987; Hahmann et al., 2013). GIS integrate many different kinds of data layers using spatial location. Most data have a geographic component. GIS data includes imagery, features and base maps linked to spreadsheets and tables. Spatial analysis lets you evaluate suitability and capability, estimate and predict, interpret and understand and much more, lending new perspectives to your insight and decision-making. Apps provide focused user experiences for getting work done and bringing GIS to life for everyone. GIS apps work virtually everywhere: on your mobile phones, tablets, in web browsers and on desktops. The geographic information (latitude and longitude) associated with the infection rates among susceptible species of fresh water fish was recorded and built a geo-dataset for GIS development. Therefore, we conduct the survey on metacercarial infections in the second intermediate host from north and northeast of Thailand and Mekong region of Myanmar. Moreover, investigation of metacercarial infections in the second intermediate host can provide a more valuable information on the trematode epidemiology. We also investigate the geographic information of the prevalence of Fish borne trematode matacercariae infections in freshwater cypinoids fishes from Mekong region of Myanmar, Central region of Myanmar, north of Thailand and northeast of Thailand. The geographic information (latitude and longitude) associated with the infection rates among susceptible species of fresh water fish was recorded and used to build a geographical information system. A number of environmental parameters such as population density were imported to system as well. The development of GIS can be useful in establishing a prevention strategy for the transmission of food borne diseases from infected fish in water catchment areas.

1.2 Research Objectives

1.2.1 To observe the current status of fish borne parasitic zoonosis infections in lower Mekong region.

1.2.2 To conduct the survey on metacercarial infections in the second intermediate host from north and northeast of Thailand and Myanmar

1.2.3 To establish surveillance model of fish- borne parasites by using Geographic Information System

CHAPTER 2

LITERATURE REVIEW

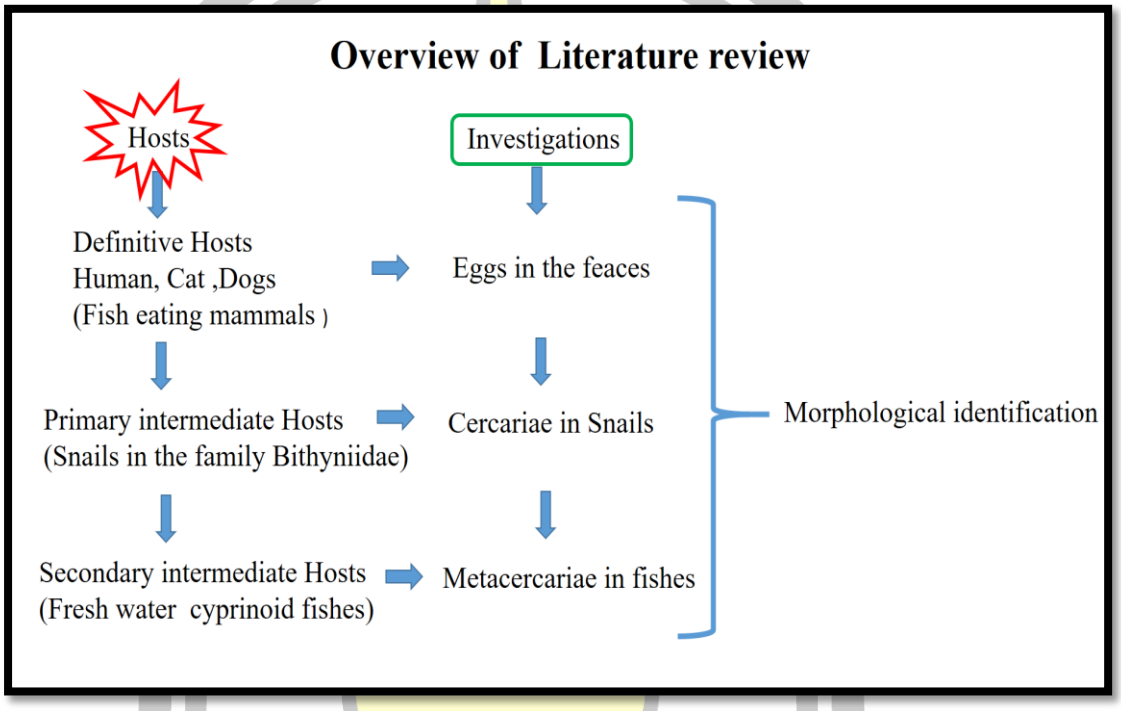


Figure 2 overview illustration of literature review

2.1 Classification of human parasites

(Dorny et al., 2009)

In the classification of human parasite (Table), there are two main categories which are protozoa (Unicellular) and helminthes (Multicellular).

The helminthes are divided into

1. Nematodes (round worms) occurs in intestine, blood and tissue
2. Cestodes (tapeworms) that can be found in intestine and tissue
3. Trematodes (flukes/flatworms) can be occurred in liver, lungs, intestine and blood.

The Protozoa: single cell for all functions are amebae, flagellaes, ciliates and apicomplexan

Table 1 classification of human parasite

Helminths (multicellular)	Protozoa (Unicellular)
Nematodes (roundworms) - Intestinal - Blood and tissue	Intestinal - Amebae - Flagellates - Ciliates - Apicomplexa
Cestodes (tapeworms) - Intestinal - Tissue	Blood/tissue - Amebae - Flagellates - Apicomplexa
Trematodes (flukes/flatworms) - Intestinal - Liver/Lung - Blood	

2.2 Classification of fish borne parasites

(Lima dos Santos and Howgate, 2011)

Trematodes-especially species of the families Opisthorchiidae and Heterophyidae; Nematodes - mostly species of the families Anisakidae and Gnathostomatidae; Cestodes - species of the family Diphyllobothriidae.

2.3 Trematodes

Among the classification of human parasites, trematodes infections are the most important emerging fish-borne parasites. The disease caused by trematode parasite is called trematodiasis. Particularly, small liver flukes, Opisthorchiidae or minute intestinal flukes (MIF), Heterophyidae are most common fish borne trematodes parasite infection in Asia and South East Asia courntries (Jeon et al., 2012). Opisthorchiidae: *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis*

sinensis are most medically important fish borne parasite in the globe (King and Sckolz, 2001).

Heterophyidae, a small sized fluke, about 1 mm. in length, and is parasitic mostly found in the small intestine. One species of fish can be contaminated with more than one species of fish- borne Trematodes metacercaria (figure). Opisthorchis liver flukes and heterophyid intestinal flukes are highly prevalent fish- borne Trematodes in Southeast Asian countries. Fish-borne trematodiasis caused by liver or minute intestinal flukes (MIF) can cause chronic diseases. Therefore, awareness should be placed on these infections as an important public health issue.

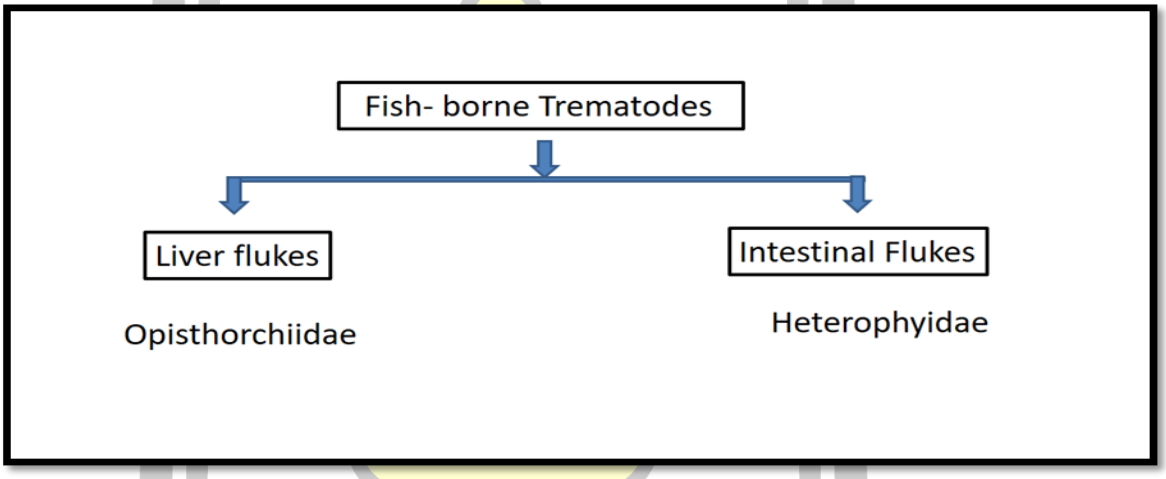


Figure 3 Common Fish- borne Trematodes parasites

2.4 *Opisthorchis viverrini*

2.4.1 Taxonomic information (WHO, 1995)

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Trematoda

Subclass: Digenea

Order: Opisthorchiida

Family: Opisthorchiidae

Genus: Opisthorchis

Species: *Opisthorchis viverrini*

2.4.2 Classification and Geographical distribution of Opisthorchiidae

Table 2 Classification and Geographical distribution of Opisthorchiidae

Species	Country
Opisthorchis viverrini	Cambodia , Lao PDR , Thailand , Central and South Vietnam , Myanmar
Opisthorchis felineus	The Baltic States, eastern Germany, Italy , Kazakhstan , Poland, Russia , Eastern Siberia, Ukraine
Clonorchis sinensis	China ,Japan ,Korea , Russia ,Thailand , North Vietnam
Opisthorchis noverca	India
Metorchis bilis	Russia
Metorchis conjunctus	Canada, Greenland
Metorchis orientalis	China
Amphimerus sp.	Ecuador

2.4.3 History of *Opisthorchis viverrini*

O. viverrini was first discovered in an Indian fishing cat (Prionailurus viverrus), originally from Southeast Asia in 1886. The first human specimen was described by a British parasitologist Robert Thomson Leiper in 1915 and the specimen was collected them from the postmortem examination of two prisoners at a jail in Chaing Mai, northern Thailand. It was in 1955 when Elvio H. Sadun from the U. S. Public Health Service analyzed the cases of opisthorchiasis in Thailand and concluded that all the infections were due to *O. viverrini* (Sadun, 1955)

2.4.4 Morphology of *O viverrini* (metacercaria)

Metacercariae of all opisthorchiid species have similar morphology.The cyst is oval, there are metacercarial body folds, and the metacercariae move actively (Boonmekam et al., 2017). They have oral and ventral suckers, the caeca and excretory bladder can clearly be seen; and other organs have not yet developed (Figure).

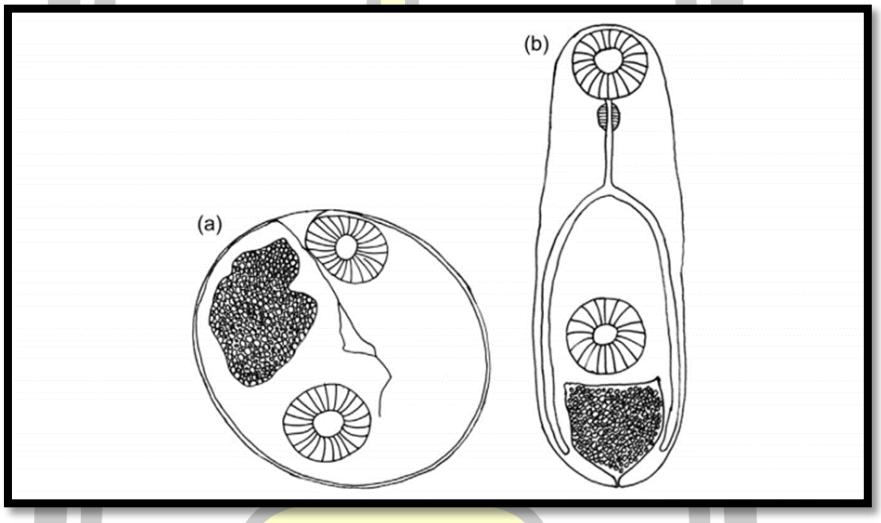


Figure 4 Metacercariae of *Opisthorchis viverrini*

(a) Encysted metacercariae of *Opisthorchis viverrini*
(b) Excysted metacercariae of *Opisthorchis viverrini*

2.4.5 Life cycle of *O viverrini* (Young et al., 2014)

Starting from a human host, the adult worms deposit fully developed eggs that are passed in the feces, the eggs from adult flukes are passed out with the faeces. The eggs must get into water and be able to infect their first intermediate host, a freshwater snail. After being ingested by a suitable snail, the eggs release miracidia which undergo in the snail several developmental stages: sporocysts, rediae, cercariae. The snail intermediate hosts are Bithynia goniompharus, B funiculata and B siamensis. Cercariae are released from the snail and then penetrate freshwater fish that are the second intermediate host (Cyclocheilichthys spp., Puntius spp., Hampala dispa), encysting as metacercariae in the muscles or under the scales. Cats, dogs, and

various fish-eating mammals including humans are the definitive host. They become infected by ingesting undercooked fish containing infective metacercariae. In infected definitive host, the metacercaria excyst in the duodenum and ascend through the ampulla of Vater into the biliary ducts, where they attach and develop to adults, which lay eggs after 3 to 4wk. The adult worms reside in the biliary system of the mammalian host, where they attach to the mucosa.

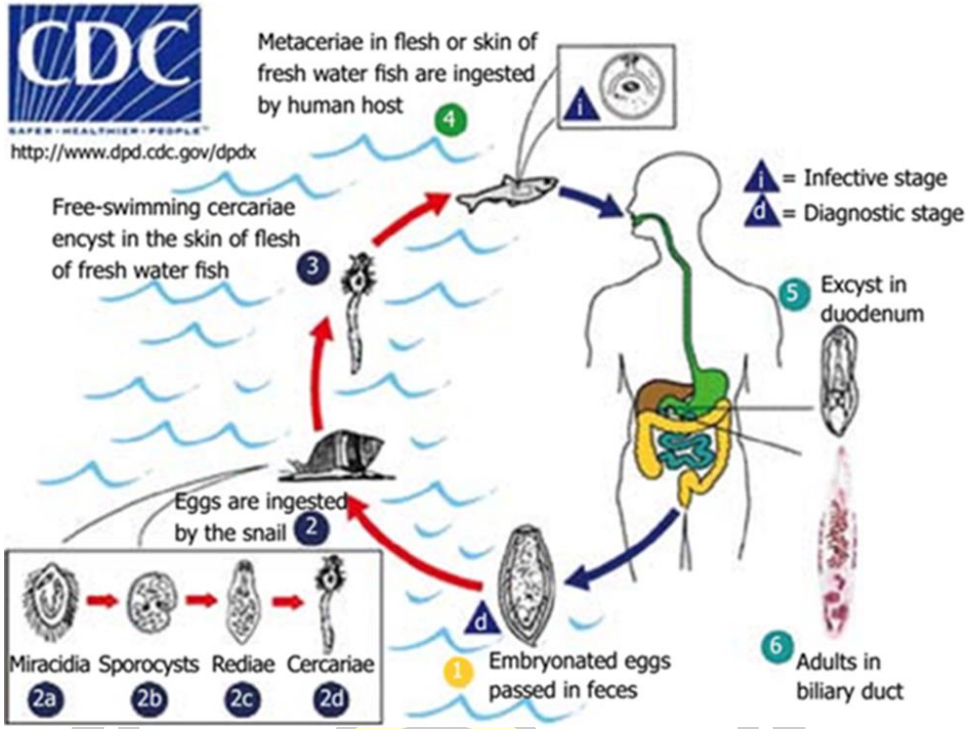


Figure 5 Life cycle of *O viverrini* (Young et al., 2014)

2.4.6 First intermediate host

The first intermediate hosts include freshwater snails of the genus Bithynia. The only known host is Bithynia siamensis (that include all its three subspecies). Snails are infected by the free-swimming larvae called miracidia in water bodies where faecal matters of infected mammals are deposited. Inside the snail tissue, the miracidia grow into sposocysts, that contain spore-like daughter cells. The daughter cells called rediae that multiply and develop into numerous larvae called cercariae. Each cercaria has a large head and a long tail. The cercariae escape from

the snail and enter the water body again as free-swimming larvae. Their tails act as a propeller for swimming and they actively search for a fish host.

2.4.7 Second intermediate host

The cercaria then locates a cyprinoid fish, encysts in the fins, skin and musculature of the fish, and becomes a metacercaria. Habitats of second intermediate hosts of *Opisthorchis viverrini* include freshwater habitats with stagnant or slow-moving waters (ponds, river, aquaculture, swamps, rice fields). In 1965 there were known 9 fish hosts of *O. viverrini*. Up to 2002 there were known 15 species of fishes from 7 genera of the family Cyprinidae, that serve as second intermediate host (Prakobwong, Suwannatrai, Sancomerang, Chaipibool, & Siriwechtumrong, 2017).

2.4.8 Definitive host

The finished dish of koi pla made of raw fish accompanied by rice and vegetables. This dish is a dietary staple of many northeastern Thai villagers and is a common source of infection with *Opisthorchis viverrini*. The metacercarial stage is infective to humans and other fish-eating mammals including dogs, cats, rats, and pigs. Fish contain more metacercaria from September to February, before the dry season and this is the period, when humans are usually infected. Infection is acquired when people ingest raw or undercooked fish. Dishes of raw fish are common in the cuisine of Laos and the cuisine of Thailand: koi-pla, raw fish in spicy salad larb-pla, salted semi-fermented fish dishes called pla-ra (pla ra), pla som and som fak. The natural definitive host is the leopard cat (Prionailurus bengalensis). The young adult worm escapes from the metacercarial cyst in the upper small intestine and then migrates through the ampulla of Vater into the biliary tree, where it develops to sexual maturity over four to six weeks, thus completing the life cycle. The adult worms primarily live in the bile duct, gall bladder, and sometimes in the pancreatic duct.

2.5 Heterophyid Infections

2.5.1 Taxonomic information (Stewart, 1998)

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Trematoda

Subclass: Digenea

Super order: Epitheliocystida

Order: Opisthorchiida

Family: Heterophyidae

Genus: Metagonimus

Genus: Heterophyes

Genus: Centrocestus

Genus: Pygidiopsis

Genus: Stellantchasmus

Genus: Haplorchis

Genus: Procerovum

2.5.2 Classification and geographical distribution

Table 3 Classification and Geographical distribution of Heterophyidae

Species	Country
Haplorchis taichui	Bangladesh, China, Iran, Lao PDR, Pakistan, Philippines, Taiwan, Thailand, Vietnam
Haplorchis pumilio	China, Egypt, Iran, Lao PDR, Philippines, Taiwan, Thailand, Vietnam
Haplorchis yokogawai	China, Indonesia, Lao PDR, Philippines, Taiwan, Thailand, Vietnam

Table 3 (Continued)

Species	Country
Centrocestus caninus	China, Taiwan, Thailand
Centrocestus formosanus	China, Lao PDR, Philippines, Taiwan,
Centrocestus longus	China, Taiwan
Centrocestus armatus	Japan, Korea
Centrocestus cuspidatus	Egypt, Taiwan
Stellantchasmus falcatus	Hawaii, Japan, Korea, Philippines, Taiwan, Thailand, Vietnam,
Pygidiopsis summa	Japan, Korea,
Procerovum calderoni	Philippines
Procerovum varium	Japan
Heterophyes dispar	Korea
Heterophyes heterophyes	Egypt, Iran, Israel, Tunisia, Turkey
Heterophyes kutsuradai	Japan
Heterophyes nocens	Japan, Korea
Heterophyopsis continua	China, Japan, Korea
Metagonimus minutus	China, Taiwan
Metagonimus niyatai	Japan, Korea
Metagonimus takahashii	Korea

Table 3 (Continued)

Species	Country
Metagonimus yokogawai	China, East Indies, Indonesia, Iran, Japan, Korea, Philippines, Taiwan , Rumania ,Russia ,Spain, Ukraine
Stictodora fuscata	Japan, Korea
Ascocotyle longa	Brazil
Apophallus donicus	Oregon-USA

2.5.3 History of Heterophyidae

Ohdner named the family Heterophyidae in 1914, combining a set of previous genera which have since been regarded as morphologically similar. He divided the family into five distinct sub-families which have been greatly adjusted over time. Many of the original genera have been subsequently excluded because of morphological differences unknown at the time and new ones included in the family.

2.5.4 Morphology of Metacercaria Stage of heterophyes

Metacercaria of Heterophyid is embedded in a double cyst wall layer. The cysts are sub spherical shape. The outer cyst wall is thick with stellate melanin pigment granules and the inner cyst wall is thin .The encysted metacercaria is round , sized 200-280 µm in diameter (Shameen, Madhavi, 1988). The excysted metacercaria is elongated shape, sized 334-362 µm in length and 90-96 µm in width with dispersed eye spot pigment in fore body region (Scholz et al., 2019). The tegument is covered with numerous spines. Oral sucker well developed .The ventral sucker is armed with spines arranged in 3 groups, in ventro-genital sac (Figure)

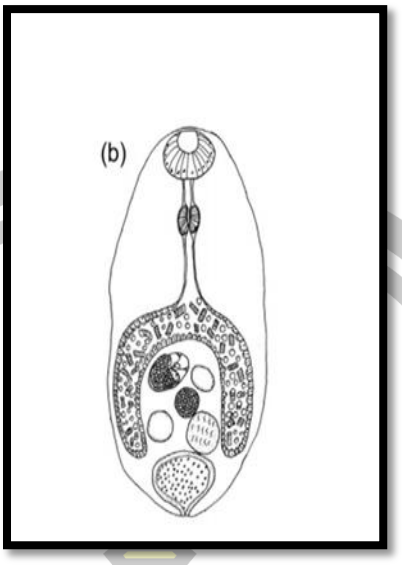


Figure 6 Morphology of Metacercaria Stage of heterophyes

2.5.5 Life cycle of heterophyes

The Adult flukes live in the small intestine of mammals and released embryonated eggs each with a fully-developed miracidium, and eggs are passed in the host's feces. After ingestion by a suitable snail (first intermediate host), the eggs hatch and release miracidia which penetrate the snail's intestine Genera Cerithidia and Pironella are important snail hosts in Asia and the Middle East respectively. The miracidia undergo several developmental stages in the snail, i.e. sporocysts. The number, rediae and cercariae. Many cercariae are produced from each redia. The cercariae are released from the snail. The number 3 and encyst as metacercariae in the tissues of a suitable fresh/brackish water fish (second intermediate host). The definitive host becomes infected by ingesting undercooked or salted fish containing metacercariae. After ingestion, the metacercariae excyst, attach to the mucosa of the small intestine and mature into adults (measuring 1.0 to 1.7 mm by 0.3 to 0.4 mm). In addition to humans, various fish-eating mammals (e.g., cats and dogs) and birds can be infected by heterophyes .

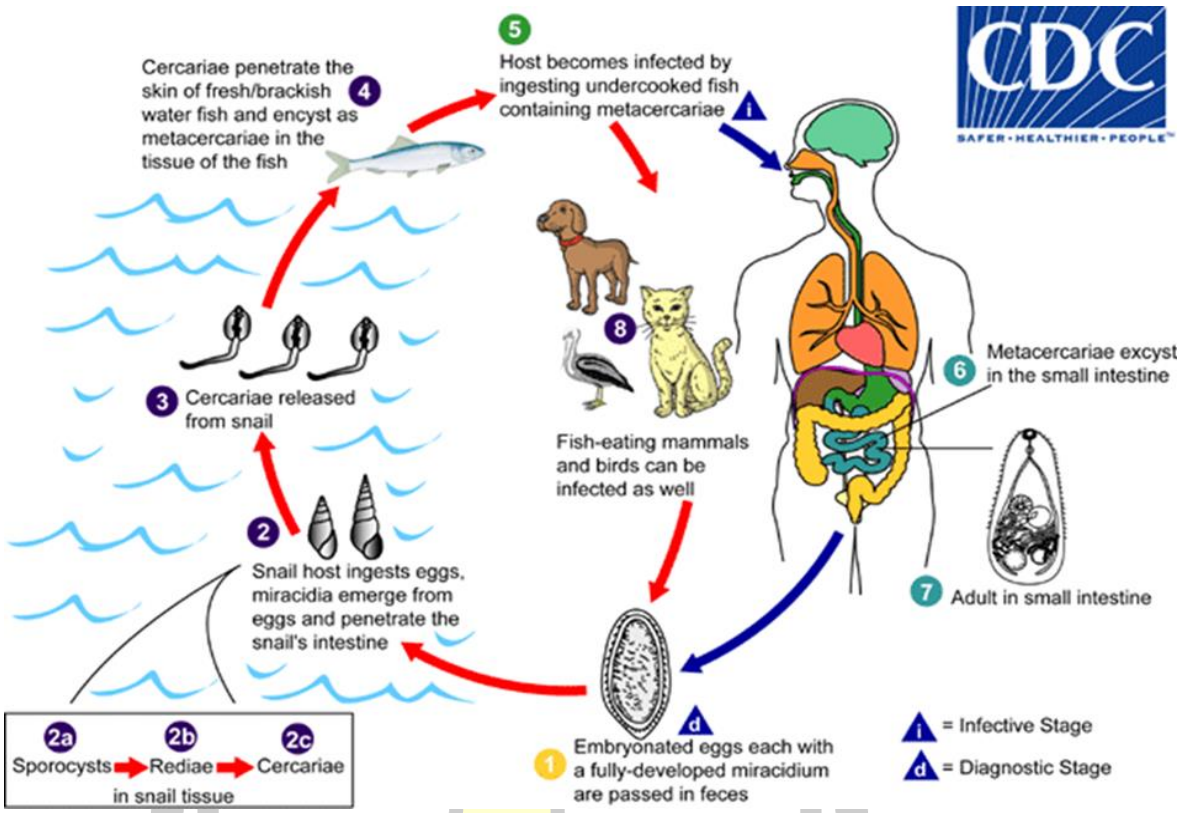


Figure 7 Life cycle of Heterophyes

2.6 Species of cyprinoid fishes that found in Mekong basin countries

Table 4 Species of cyprinoid fishes that found in Mekong basin countries

No	Location	Species of cyprinoid fishes	Species of metacercariae	References
1.	MaeNgud reservoir, ChiangMai Province	*Thynnichthys thynnoides* *Puntioplites proctozysron* *Hampala macrolepidota* *Puntius leiacanthus* *Puntius gonionotus*	*Haplorchis spp* *H. taichui*	(Sukontason et al., 2001)

Table 4 (Continued)

No	Location	Species of cyprinoid fishes	Species of metacercariae	References
2.	Southern Cambodia	*Barbodes altus* *Cyclocheilichthys Apagon* *Cyclocheilichthys enoplos* *Hampala dispar* *Hampala macrolepidota* *Henicorhynchus siamensis* *Puntioplites proctozysron* *Puntius Brevis* *Systomus orphoides* *Thynnichthys thynnoides*	*Opisthorchis viverrini*	(Touch, Komalamisra, Radomyos, & Waikagul, 2009)
3.	20 provinces in northeastern Thailand	*Cyclocheilichthys armatus*, *Puntius orphoides*, *Hampala dispar*, *Henicorhynchus siamensis*, *Osteochilus hasselti*, *Puntioplites proctozysron*	*Opisthorchis viverrini*	(Pinlaor et al., 2013)
4.	Yangon , Myanmar	*Thynnichthys thynnoides* *Chelon macrolepis* *Punitus aurotaeniatus* *Esomus Altus* *Channa striata* *Rhynogobius sp.* *Anabus testudineus* *Trichogaster pectoralis* *Mystacolecucus sp.* *Notopterus notopterus* *Labeo sp.* *Puntioplites proctozysron*	*Haplorchis taichui*, *H. pumilio*, *H.yokogawai*, *Centrocestus spp.*, *Stellantchasmus falcatus*, *Pygidiopsis cambodiensis*, *Procerovum sp*	(J. Y. Chai et al., 2017)

Table 4 (Continued)

No	Location	Species of cyprinoid fishes	Species of metacercariae	References
5.	Khammouane Province, Lao PDR	*Cyclocheilichthys repasson* *C. armatus* *C. enoplos* *Dangila lineata* *Henicorhynchus lineatus* *Hampala dispar* *Puntioplites proctzysron* *Osteochilus waandersii*	*O. viverrini metacercariae*	(Ruenwongsa et al., 2009)

2.7 List of species of metacercariae that found Mekong river basin of Southeast Asia countries

Table 5 List of species of metacercariae that found Mekong river basin of Southeast Asia countries

No	Species of metacercariae	Location , Country	Reference
1	*Opisthorchis viverrini*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al., 1976)
	Opisthorchis viverrini	Vientiane Municipality and Savannakhet Province, Lao PDR	(H. Rim et al., 2008)
	Opisthorchis viverrini	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al., 2015)
	Opisthorchis viverrini	northeastern of Thailand	(Onsurathum et al., 2016)
	Opisthorchis viverrini	Phnom Penh and Pursat , Cambodia	(Chai et al., 2014)

Table 5 (Continued)

No	Species of metacercariae	Location , Country	Reference
2	*Haplorchis taichui*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al.,1991)
	Haplorchis taichui	Vientiane Municipality and Savannakhet Province, Lao PDR	(H. Rim et al., 2008)
	Haplorchis taichui	from Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al., 2015)
	Haplorchis taichui	Chiang Mai Province, Thailand	(Saenphet et al., 2008)
	Haplorchis taichui	Bo Kluea District and Pua District, Nan Province, Thailand	(Dusit et al., 2016)
	Haplorchis taichui	northeastern of Thailand	(Onsurathum et al., 2016)
	Haplorchis taichui,	Yangon, Myanmar	(Chai et al., 2017)
3	*Haplorchis pumilio*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al., 1991)
	Haplorchis pumilio	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al., 2015)
	Haplorchis pumilio	Yangon, Myanmar	(Chai et al., 2017)
	Haplorchis pumilio,	Phnom Penh and Pursat , Cambodia	(Chai et al., 2014)
4	*Haplorchis yokogawai*	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al., 2015)
	Haplorchis yokogawai	Vientiane Municipality and Savannakhet Province, Lao PDR	(Rim et al., 2008)
	Haplorchis yokogawai	Yangon, Myanmar	(Chai et al., 2017)
	Haplorchis yokogawai	Phnom Penh and Pursat , Cambodia	(Chai et al., 2014)

Table 5 (Continued)

No	Species of metacercariae	Location , Country	Reference
5	*Haplorchoides mehrai*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al., 1991)
6	*Haplorchoides* sp.	Chiang Mai Province, Thailand	(Saenphet et al., 2008)
7	*Centrocestus formosanus*	Vientiane Municipality and Savannakhet Province, Lao PDR	(H. Rim et al., 2008)
	Centrocestus formosanus	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al., 2015)
	Centrocestus formosanus	Phnom Penh and Pursat , Cambodia	(Chai et al., 2014)
8	*Centrocestus caninus*	Chiang Mai Province, Thailand	(Saenphet et al., 2008)
9	*Centrocestus* spp.	Yangon, Myanmar	(Chai et al., 2017)
10	*Procerovum varium*	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al., 2015)
11	*Procerovum* sp.	Yangon, Myanmar	(Chai et al., 2017)
	Procerovum sp	Phnom Penh and Pursat , Cambodia	(Chai et al., 2014)
12	*Pygidiopsis cambodiensis*	Yangon, Myanmar	(Chai et al., 2017)
13	*Stellantchasmus falcatus*	Chiang Mai Province , Thailand	(Saenphet et al., 2008)
	Stellantchasmus falcatus	Yangon, Myanmar	(Chai et al., 2017)

2.8 Morphologically differential diagnosis of fish borne trematode metacercariae in Southeast Asia countries along the Mekong river basin

Table 6 Morphologically differential diagnosis of fish borne trematode metacercariae in Southeast Asia countries along the Mekong river basin

No	Species	Excretory bladder	OS: oral sucker; VS: ventral sucker	Shape	ventro-genital sac and spine	References
1	*Opisthorchis viverrini*	O-shaped excretory bladder	equal sized 2 suckers OS: oral sucker; VS: ventral sucker	elliptical		(Sanpool et al., 2018)
2	*Haplorchis taichui*	O-shaped excretory bladder		elliptical	baseball glove-shaped ventro-genital sac with 11-19 chitinous rodlets	(Rim et al., 2008)
3	*Haplorchis pumilio*	O-shaped excretory bladder		elliptical	deer horn-like minute spines arranged in 1-2 rows	(Chai et al., 2017)
4	*Haplorchoides mehrai*	a rather small excretory bladder		variable in shape,' size		(Scholz and Giboda, 1976)
5	*Haplorchis yokogawai*	O-shaped excretory bladder		elliptical or round	U-shaped ventrogenital sac with 70-74 min spines	(Rim et al., 2008)

Table 6 (Continued)

No	Species	Excretory bladder	OS: oral sucker; VS: ventral sucker	Shape	ventro-genital sac and spine	References
6	*Centrocestus formosanus*	X-shaped excretory bladder	32 circumoral spines around the oral sucker arranged in 2 rows	elliptical	32 circumoral spines	(Chai et al., 2017)
7	*Stellantchasmus falcatus*	O-shaped excretory bladder		elliptical	Brownish pigment granules scattered in the body	(Chai et al., 2017)
8	*Pygidiopsis cambodiensis*	X shaped excretory bladder.	had an oral sucker ,a pair of eyespots, ventral sucker,	elliptical,	ventrogenital sac,	(Chai et al., 2017)
9	*Procerovum sp*	D-shaped (half-moon shaped) excretory bladder with grouped granules.		elliptical	yellow brownish pigment granules scattering in body area of intestinal bifurcation	(Chai et al., 2017)

2.9 Secondary prospective study about the association of fish species (Host), metacercariae species and locations (Myanmar, North and Northeast of Thailand)

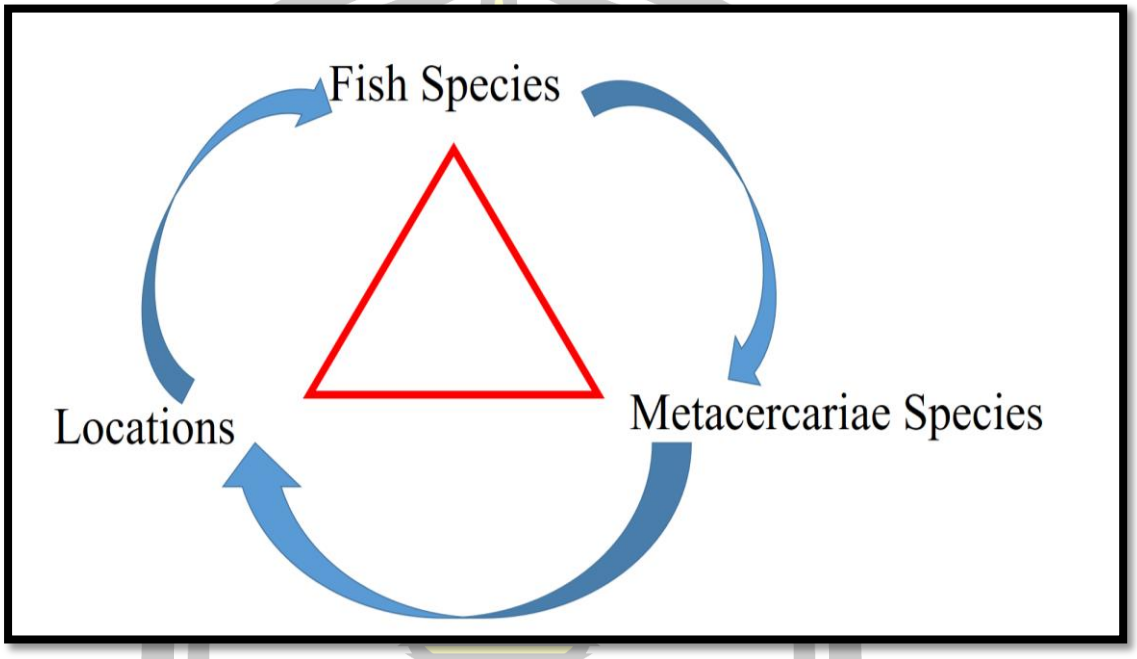


Figure 8 Conceptual frame work of the research study

2.9.1 Secondary prospective study from Myanmar

Table 7 Opisthorchis viverrini Metacercariae detected from Myanmar

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
1	*Channa Lucius*	OV	North Dagon, Yangon, Myanmar	5/5, 100%	5	Sohn et al 2019
2	*Channa striata*	OV	North Dagon,Yangon ,Myanmar	1/29, 3.5%	1	Sohn et al 2019

Table 7 (Continued)

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
3	*Anabas testudineus*	OV	North Dagon ,Yangon ,Myanmar	1/14, 7.1%	1	Sohn et al 2019
4	*Puntioplites* sp.,	OV	North Dagon ,Yangon ,Myanmar	1/15, 6.7%	1	Sohn et al 2019
5	*Puntius brevis*	OV	Bago, central Myanmar	10/30, 33.4%	10	Sanpool et al 2018

Table 8 Haplorchis taichui Metacercariae detected from Myanmar

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
1	*Thynnichthys thynnoides*	HT	Yangon, Myanmar	40/67,59.7%	2250	Chai et al 2017
2	*Puntius aurotaeniatus*	HT	Yangon, Myanmar	9/32,28.1%	79	Chai et al 2017
3	*Esomus altus*	HT	Yangon, Myanmar	1/23,4.4%	2	Chai et al 2017
4	*Mystacoleucus* sp.	HT	Yangon, Myanmar	3/10,30%	23	Chai et al 2017
5	*Labeo* sp.	HT	Yangon, Myanmar	5/5,100%	134	Chai et al 2017

Table 9 *Haplorchis pumilio* Metacercariae detected from Myanmar

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
1	*Thynnichthys thynnoides*	HP	Yangon, Myanmar	43/67,64.2%	1157	Chai et al 2017
2	*Puntius aurotaeniatus*	HP	Yangon, Myanmar	13/32,40.6%	105	Chai et al 2017
3	*Esomus altus*	HP	Yangon, Myanmar	6/23,26.1%	19	Chai et al 2017
4	*Channa striata*	HP	Yangon, Myanmar	14/20,70.0%	913	Chai et al 2017
5	*Anabas testudineus*	HP	Yangon, Myanmar	7/12,58.3%	42	Chai et al 2017
6	*Rhynogobius* sp.	HP	Yangon, Myanmar	2/17,11.8%	2	Chai et al 2017
7	*Trichogaster pectoralis*	HP	Yangon, Myanmar	9/10,90.0%	16	Chai et al 2017
8	*Mystacoleucus* sp.	HP	Yangon, Myanmar	1/10,10.0%	8	Chai et al 2017
9	*Labeo* sp.	HP	Yangon, Myanmar	1/20,20.0%	1	Chai et al 2017

2.9.2 Secondary prospective study from Northern Thailand

Table 10 Opisthorchis viverrini Metacercariae detected from North of Thailand

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
1	*Systomus stoliczkanus*	OV	Chiang Mai province, Thailand	3/18, 16.67%	30	(Wongsawad et al., 2013)

Table 10 (Continued)

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
2	*Puntius gonionotus*		Ban Pao, Chiang Mai , Thailand	Total 58	55	(Sukontason et al., 1999)
	Puntius orphoides	OV	Ban Pao, Chiang Mai , Thailand	Total 18	25	(Sukontason et al., 1999)
3	*Thynnichthys thynnoides*		Ban Pao, Chiang Mai , Thailand	Total 302	6	(Sukontason et al., 1999)
4	*Hampala macrolepidota*		Ban Pao, Chiang Mai , Thailand	Total 10	2	(Sukontason et al., 1999)
5	*Labiobarbus burmanicus*		Ban Pao, Chiang Mai , Thailand	Total 60	1	(Sukontason et al., 1999)

Table 11 *Haplorchis taichui* Metacercariae detected from North of Thailand

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
A	*Amblyrhynchichthys trutcatus*	HT	Chiang Mai province, Thailand	3/3	59	(Saenphet et al 2008)
B	*Barbodes gonionotus*	HT	Mae Ngad Reservoir	4/4,100%	54	(Nithikathkul et al 2008a)

Table 11

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
	Barbonymus gonionotus	HT	Chiang Mai province, Thailand	73/208, 35.10%	4992	(Wongsawad et al 2013)
	Barbonymus gonionotus	HT	Chiang Mai province, Thailand	30/33	2,438	(Saenphet et al 2008)
	Barbodes gonionotus	HT	Mae Kuang Udomtara Reservoir	1/1,100%	91	(Nithikathkul et al 2008a)
	Barbodes altus	HT	Mae Kuang Udomtara Reservoir	3/3,100%	48	(Nithikathkul et al 2008a)
	Barbonymus schwanenfeldi	HT	Chiang Mai province, Thailand	5/5	301	(Nithikathkul et al 2008a)
	Barbodes schwanenfeldi	HT	Nan province, Northern Thailand	4/4,100%		(Boonmekam et al 2016)
G	*Garra cambodgiensis*	HT	Nan province, Northern Thailand	12/30, 46.67%		(Boonmekam et al 2016)
H	*Hampala macrolepidota*	HT	Mae Ngad Reservoir	2/5,40%	133	(Nithikathkul et al 2008a)
	Hampala macrolepidota	HT	Chiang Mai province, Thailand	54/129 41.86%	1528	(Wongsawad et al 2013)
	Hampala macrolepidota	HT	Chiang Mai province, Thailand	14/17	35	(Saenphet et al 2008)
	Henicorhynchus siamensis	HT	Mae Ngad Reservoir	5/5,100%	542	(Nithikathkul et al 2008a)

Table 11

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
	Henicorhynchus siamensis	HT	Chiang Mai province, Thailand	103/239 43.10%	5019	(Wongsawad et al 2013)
	Henicorhynchus siamensis	HT	Mae Kuang Udomtara Reservoir	1/1,100%	4	(Nithikathkul et al 2008a)
	Henicorhynchus siamensis	HT	Chiang Mai province, Thailand	55/62	10,542	(Saenphet et al 2008)
	Hypsibarbus salweenensis	HT	Nan province, Northern Thailand	6/9, 66.67%		(Boonmekam et al 2016)
L	*Labiobarbus siamensis*	HT	Mae Ngad Reservoir	5/5,100%	164	(Nithikathkul et al 2008a)
	Labiobarbus siamensis	HT	Chiang Mai province, Thailand	54/60,	3609	(Saenphet et al 2008)
	Labiobarbus siamensis	HT	Mae Kuang Udomtara Reservoir	5/7,71.4%	1	(Nithikathkul et al 2008a)
	Labiobarbus siamensis	HT	Chiang Mai province, Thailand	86/212 40.57%	3796	Wongsawad et al 2013
M	*Mystacoleucus marginatus*	HT	Mae Ngad Reservoir	1/1,100%	11	(Nithikathkul et al 2008a)
	Mystacoleucus marginatus	HT	Mae Kuang Udomtara Reservoir	4/9,44.4%	45	(Nithikathkul et al 2008a)
	Mystacoleucus marginatus	HT	Chiang Mai province, Thailand	233/532 43.80%	7448	(Wongsawad et al 2013)

Table 11 (Continued)

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
	Mystacoleucus marginatus	HT	Chiang Mai province, Thailand	36/42	1,329	(Saenphet et al 2008)
	Mystacoleucu smarginatus	HT	Nan province, Northern Thailand	17/17, 100%		(Boonmekam et al 2016)
N	*Notopterus notopterus*	HT	Mae Kuang Udomtara Reservoir	1/2,50%	1	(Nithikathkul et al 2008a)
O	*Osteochilus hasselti*	HT	Mae Kuang Udomtara Reservoir	5/5,100%	19	(Nithikathkul et al 2008a)
	Osteochilus hasselti	HT	Chiang Mai province, Thailand	96/184 52.17	1184	(Wongsawad et al 2013)
	Osparus puchellus	HT		35/38, 92.41%		(Boonmekam et al 2016)
P	*Puntioplites proctozysron*	HT	Mae Ngad Reservoir	6/6,100%	23	(Nithikathkul et al 2008a)
	Puntioplites proctozysron	HT	Mae Kuang Udomtara Reservoir	1/1,100%	2	(Nithikathkul et al 2008a)
	Puntioplites proctozysron	HT	Chiang Mai province, Thailand	31/37	751	(Saenphet et al 2008)
	Paralaubuca harmandi	HT	Chiang Mai province, Thailand	2/2	48	(Saenphet et al 2008)
	Poropuntius normani	HT	Mae Kuang Udomtara Reservoir	3/3,100%		(Nithikathkul et al 2008a)

Table 11 (Continued)

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
	Poropuntius deauratus	HT	Nan province, Northern Thailand	1/1,100%		(Boonmekam et al 2016)
R	*Ratamus guttatus*	HT	Chiang Mai province, Thailand	1/1	745	Saenphet et al 2008
S	*Systomus orphoides*	HT	Mae Ngad Reservoir	1/1,100%	20	(Nithikathkul et al 2008a)
	Systomus orphoides	HT	Chiang Mai province, Thailand	45/54	1,520	Saenphet et al 2008
	Systomus orphoides	HT	Chiang Mai province, Thailand	74/82 90.24	826	Wongsawad et al 2013
	Systomus orphoides	HT	Nan province, Northern Thailand	1/1,100%		(Boonmekam et al 2016)
	Scaphiodonicthys acanthopterus	HT	Nan province, Northern Thailand	9/17, 52.94%		(Boonmekam et al 2016)
	Sytsomus stolitzkaenus	HT	Nan province, Northern Thailand	16/32, 50%		(Boonmekam et al 2016)

Table 12 *Haplorchis pumilio* Metacercariae detected from North of Thailand

No	Fish Species	Metacercariae	Location	Prevalence	Number of Metacercariae	Reference
1	*Barbodes gonionotus*	HP	Chom Thong district, Chiang Mai province, Thailand	25/25	4	(Kumchoo et al 2005)
2	*Henicorhynchus siamensis*	HP	Chom Thong district, Chiang Mai province, Thailand	91/93	4	(Kumchoo et al 2005)
3	*Henicorhynchus siamensis*	HP	Mae Taeng district, Chiang Mai province, Thailand	57/60	2	(Kumchoo et al 2005)
4	*Labiobarbus siamensis*	HP	Mae Taeng district Chiang Mai province, Thailand	46/48	1	(Kumchoo et al 2005)

Table 13 *Haplorchoides* sp. Metacercariae detected from North of Thailand

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
A	*Anabas testudineus*	HAP	Mae Kuang Udomtara Reservoir	1/1,100%	2	(Nithikathkul et al 2008a)
	Amblyrhynchichthys trutcatus	HAP	Chiang Mai province, Thailand	3/3	57	(Saenphet et al 2008)

Table 13 (Continued)

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
B	*Barbodes gonionotus*	HAP	Mae Ngad Reservoir	4/4,100%	6	(Nithikathkul et al 2008a)
	Barbonymus gonionotus	HAP	Chiang Mai province, Thailand	135/208, 64.90%	3128	(Wongsawad et al 2013)
	Barbodes gonionotus	HAP	Mae Kuang Udomtara Reservoir	1/1,100%	4	(Nithikathkul et al 2008a)
	Barbonymus gonionotus	HAP	Chiang Mai province, Thailand	30/33	3,179	(Saenphet et al 2008)
	Barbodes altus	HAP	Mae Kuang Udomtara Reservoir	3/3,100%	3	(Nithikathkul et al 2008a)
	Barbonymus schwanenfeldi	HAP	Chiang Mai province, Thailand	5/5	280	(Saenphet et al 2008)
C	*Cyclocheilichthys apagon*	HAP	Mae Kuang Udomtara Reservoir	1/1,100%	59	(Nithikathkul et al 2008a)
H	*Hampala macrolepidota*	HAP	Mae Ngad Reservoir	2/5,40%	24	(Nithikathkul et al 2008a)
	Hampala macrolepidota	HAP	Chiang Mai province, Thailand	75/129 58.14%	2322	(Wongsawad et al 2013)
	Hampala macrolepidota	HAP	Chiang Mai province, Thailand		243	(Saenphet et al 2008)
	Henicorhynchus siamensis	HAP	Mae Ngad Reservoir	5/5,100%	60	(Nithikathkul et al 2008a)
	Henicorhynchus siamensis	HAP	Chiang Mai province, Thailand	136/239 56.90	2899	(Wongsawad et al 2013)

Table 13 (Continued)

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
	Henicorhynchus siamensis	HAP	Chiang Mai province, Thailand	55/62	7,346	(Saenphet et al 2008)
L	*Labiobarbus siamensis*	HAP	Mae Ngad Reservoir	5/5,100%	3	(Nithikathkul et al 2008a)
	Labiobarbus siamensis	HAP	Chiang Mai province, Thailand	126/212 59.43	2783	(Wongsawad et al 2013)
	Labiobarbus siamensis	HAP	Chiang Mai province, Thailand	54/60	7,327	(Saenphet et al 2008)
	Labiobarbus siamensis	HAP	Mae Kuang Udomtara Reservoir	5/7,71.4%	5	(Nithikathkul et al 2008a)
M	*Mystacoleucus marginatus*	HAP	Chiang Mai province, Thailand	299/532 56.20	4788	(Wongsawad et al 2013)
	Mystacoleucus marginatus	HAP	Chiang Mai province, Thailand	36/42	9,334	(Saenphet et al 2008)
	Mystacoleucus marginatus	HAP	Mae Kuang Udomtara Reservoir	4/9,44.4%	40	(Nithikathkul et al 2008a)
	Morulius chrysophykadian	HAP	Mae Ngad Reservoir	1/3,33.3%	1	(Nithikathkul et al 2008a)
	Puntioplites proctozysron	HAP	Mae Ngad Reservoir	6/6,100%	3	(Nithikathkul et al 2008a)
S	*Systomus orphoides*	HAP	Mae Ngad Reservoir	1/1,100%	1	(Nithikathkul et al 2008a)
	Systomus orphoides	HAP	Chiang Mai province, Thailand	45/54	10,920	(Saenphet et al 2008)

Table 13 (Continued)

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
	Systomus stoliczkanus	HAP	Chiang Mai province, Thailand	18/18 100.00	216	(Wongsawad et al 2013)
	Systomus stoliezkei	HAP	Chiang Mai province, Thailand	1/2	747	(Saenphet et al 2008)
O	*Osteochilus hasselti*	HAP	Mae Kuang Udomtara Reservoir	5/5,100%	47	(Nithikathkul et al 2008a)
	Osteochilus hasselti	HAP	Chiang Mai province, Thailand	88/184 47.83	2024	(Wongsawad et al 2013)
P	Puntioplites proctozysron	HAP	Mae Kuang Udomtara Reservoir	1/1,100%	178	(Nithikathkul et al 2008a)
	Puntioplites proctozysron	HAP	Chiang Mai province, Thailand	31/37	2,361	(Saenphet et al 2008)
	Raiamas guttatus	HAP	Chiang Mai province, Thailand	8/8,100%	48	(Wongsawad et al 2013)
T	*Tricopsis vittatus*	HAP	Chiang Mai province, Thailand	23/38	147	(Saenphet et al 2008)
	Tricogaster microlepis	HAP	Chiang Mai province, Thailand	13/15		(Saenphet et al 2008)
R	*Rasbora parviei*	HAP	Chiang Mai province, Thailand	26/31	29	(Saenphet et al 2008)
	Rasbora tornieri	HAP	Chiang Mai province, Thailand	1/3	1	(Saenphet et al 2008)

Table 13 (Continued)

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
O	*Osteochilus hasselti*	HAP	Chiang Mai province, Thailand	1/2	278	(Saenphet et al 2008)

2.9.3 Secondary prospective study from Northeast of Thailand

Table 14 *Opisthorchis viverrini* Metacercariae detected from North of Thailand

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
1	*Henicorhynchus siamensis*	OV	Udon Thani province, Northeast	219/946 23.1%	5672	(Prakobwong et al 2017)
2	*Cyclocheilichthys repasson*	OV	Udon Thani province, Northeast	134/843 15.8%	2063	(Prakobwong et al 2017)
3	*Hampala dispar*	OV	Udon Thani province, Northeast	15/276 5.4%	36	(Prakobwong et al 2017)
4	*Barbonymus gonionotus*	OV	Udon Thani province, Northeast	3/124 16.9%	3	(Prakobwong et al 2017)
5	*Cyclocheilichthys armatus*	OV	Yasothon, Northeast	Total-193	9	(Pinlaor et al 2013)
6	*Cyclocheilichthys armatus*	OV	Ubon Ratchathani, Northeast	Total-277	4	(Pinlaor et al 2013)
7	*Puntius orphoides*	OV	Nakhon Ratchasima, Northeast	Total-639	33	(Pinlaor et al 2013)

Table 14 (Continued)

No	Fish Species	metacercariae	Location	Prevalence	Number of Metacercariae	Reference
8	*Cyclocheilichthys armatus*	OV	Sakon Nakhon, Northeast	Total-181	14	(Pinlaor et al 2013)
9	*Cyclocheilichthys armatus*	OV	Si Sa Ket, Northeast	Total-758	2580	(Pinlaor et al 2013)
10	*Cyclocheilichthys armatus*	OV	Surin, Northeast	Total-345	16	(Pinlaor et al 2013)
11	*Hampala dispar*	OV	Surin, Northeast	Total-55	3	(Pinlaor et al 2013)
12	*Cyclocheilichthys armatus*	OV	Buri Ram, Northeast	Total-41	12	(Pinlaor et al 2013)
13	*Hampala dispar*	OV	Amnat Charoen, Northeast	Total-8	52	(Pinlaor et al 2013)
14	*Henicorhynchus siamensis*	OV	Mukdahan, Northeast	Total-802	591	(Pinlaor et al 2013)
15	*Hampala dispar*	OV	Khon Kaen, Northeast	Total-10	35	(Pinlaor et al 2013)
16	*Cyclocheilichthys armatus*	OV	Chaiyaphum, Northeast	Total-197	330	(Pinlaor et al 2013)
17	*Osteochilus hasselti*	OV	Nakhon Phanom	Total -23	98	(Pinlaor et al 2013)
18	*Puntioplites proctozysron*	OV	Nong Khai	Total-83	94	(Pinlaor et al 2013)

2.10 Carcinogenic parasite

Carcinogenic parasite is a parasitic organism which depends on other organisms (called hosts) for their survival, and cause cancer in such hosts. Medically-proven carcinogenic parasites are three species of flukes (trematodes), namely the urinary blood fluke (Schistosoma haematobium), the Southeast Asian liver fluke (*Opisthorchis viverrini*) and the Chinese liver fluke (Clonorchis sinensis). S. haematobium is prevalent in Africa and the Middle East, and is the leading cause of bladder cancer (only next to tobacco smoking). *O. viverrini* and C. sinensis are both found in eastern and southeastern Asia, and are responsible for cholangiocarcinoma (cancer of the bile ducts). The International Agency for Research on Cancer declared them in 2009 as a Group 1 biological carcinogens in humans (Bouvard et al., 2009).

Group 1 carcinogens in human

Three flukes, urinary blood fluke (*Schistosoma haematobium*), Southeast Asian liver fluke (*Opisthorchis viverrini*) and Chinese liver fluke (*Clonorchis sinensis*) are classified as Group 1 carcinogens, i.e. they are substantiated and directly cancer-causing agents (Bouvard et al., 2009).

O. viverrini is a food-borne liver fluke that mainly attacks the area of the bile duct. Infection with the parasite, called opisthorchiasis is the major cause of cholangiocarcinoma, a cancer of the bile ducts, in northern Thailand, the Lao People's Democratic Republic, Vietnam and Cambodia. (Dao et al., 2016).

Cholangiocarcinoma (CCA) is a major health problem in Thailand particularly in the northeastern and northern regions. It is also known to be one of the most common causes of cancer related to death in Thailand and it has been reported that Thailand is the highest incident of the world (Green et al., 1991) (Sripa et al., 2007) (Shin et al., 2010). It has an annual incidence rate of 1–2 cases per 100,000 in the Western world, but rates of CCA have been rising worldwide over the past several decades (Landis et al., 1998). CCA is a neoplasm that involves the epithelial cells of the bile duct. CCA is originated in the bile duct in which drained bile from the liver into the small intestine. This disease is difficult to have early diagnosis, as most symptoms present late in the disease course. In addition, the specific anatomic

position can cause periductal extension and result in a very low radical excision rate and a very poor prognosis. Furthermore, CCA is considered to be an incurable and rapidly lethal disease unless all the tumors can be fully resected.

Three-year survival rates of 35% to 50% are achieved only in a subset of patients who have negative histological margins at the time of surgery (Akamatsu et al 2011). Survival of CCA patients in northeastern Thailand after supportive treatment was reported and indicated that the stage of disease was an important prognosis factor affecting survival of CCA patients who had diagnosis in late stage. To encourage patients to see health personnel at early stage is very important (Thunyaharn et al 2013). Palliative therapeutic approaches, consisting of percutaneous and endoscopic biliary drainage, have usually been used for these patients because there is no effective chemotherapeutic treatment for this type of cancer. The high mortality rate of CCA was reported in the northeast areas where found frequently of *Opisthorchis viverrini* infection (Sripa et al 2011). Recently, *O. viverrini* has been classifid as Type 1 carcinogens by the International Agency for Research on Cancer, World Health Organization (WHO) (IARC, 1994). Mortality rate of liver cancer and O. viverrini infection rate in different regions of Thailand has been reported.

Worldwide incidence (cases/100,000) of CCA was reported, The data are relative to the period between 1977 to 2007, indicates that Thailand has a highest CCA especially in the Northeast (85.00 per 100,000), North (14.6.00 per 100,000), Central (14.4 per 100,000), and South (5.7 per 100,000), respectively (Bragazzi et al 2012).

O. viverrini has three successive host for its life cycle – the first intermediate hosts are freshwater snails of the genus Bithynia, the second intermediate hosts are different cyprinid fish, and humans are the definitive hosts (King et al., 2001). Generally opisthorchiasis due to *O. viverrini* is harmless without any clinical symptoms, but in rare cases, cholangitis, cholecystitis, and cholangiocarcinoma can develop. *O. viverrini* invades the bile ducts and, rarely, the gall bladder and pancreatic duct. Heavy infection can produce problems such as fibrosis in the liver, gall bladder and bile ducts (Hitanant et al., 1987). Pathological effects on the bile ducts including inflammation, epithelial desquamation, goblet cell metaplasia, epithelial and adenomatous hyperplasia and periductal fibrosis collectively promote

cholangiocarcinoma (Pairojkul et al., 1991). Though it is not immediately life-threatening, cancer develops after 30–40 years, and the ensuing death is rapid—within 3–6 months of diagnosis (Sripa et al., 2007)

.

2.11 Geographic Information Systems (GIS)

(William et al., 1987; Hahmann et al., 2013).

Geographic Information Systems (GIS) store, analyze, and visualize data for geographic positions on Earth's surface. GIS is a computer-based tool that examines spatial relationships, patterns, and trends. By connecting geography with data, GIS better understands data using a geographic context.

The 4 main ideas of Geographic Information Systems (GIS) are:

1. Create geographic data.
2. Manage it in a database.
3. Analyze and find patterns.
4. Visualize it on a map.

Because viewing and analyzing data on maps impacts the understanding of data, it can make better decisions using GIS. It helps to understand what is where. The analysis becomes simple. Answers become clear.

Every day, GIS powers millions of decisions around world. It makes a big impact in our life and you might not even realize. For example, we use GIS for:

1. Pinpointing new store locations
2. Reporting power outages
3. Analyzing crime patterns
4. Routing in car navigation
5. Forecasting and predicting weather

Components of Geographic Information Systems

The 3 main components of Geographic Information Systems are:

1. DATA: GIS stores location data as thematic layers. Each data set has an attribute table that stores information about the feature. The two main types of GIS data are raster and vector:

RASTER

Raster look like grids because they store data in rows and columns. They can be discrete or continuous. For example, we often represent land cover, temperature data and imagery as raster data.

VECTOR

Vectors are points, lines and polygons with vertices. For example, fire hydrants, contours and administrative boundaries are often vectors.

2. HARDWARE: Hardware runs GIS software. It could be anything from powerful servers, mobile phones or a personal GIS workstation. The CPU is your workhorse and data processing is the name of the game. Dual monitors, extra storage, and crisp graphic processing cards are must-haves too in GIS.

3. SOFTWARE: ArcGIS and QGIS are the leaders in GIS software. GIS software specialize in spatial analysis by using math in maps. It blends geography with modern technology to measure, quantify and understand our world.

2.12 Drive Decisions with Spatial Analysis

These global issues need location-based knowledge that can only come from a GIS.Most people think GIS is only about “making maps”. But we harness the power of GIS because of the insights of spatial analysis. We use spatial analysis through math in maps. Spatial analysis is difficult with paper maps so that’s why we need GIS. Here are examples of spatial analysis

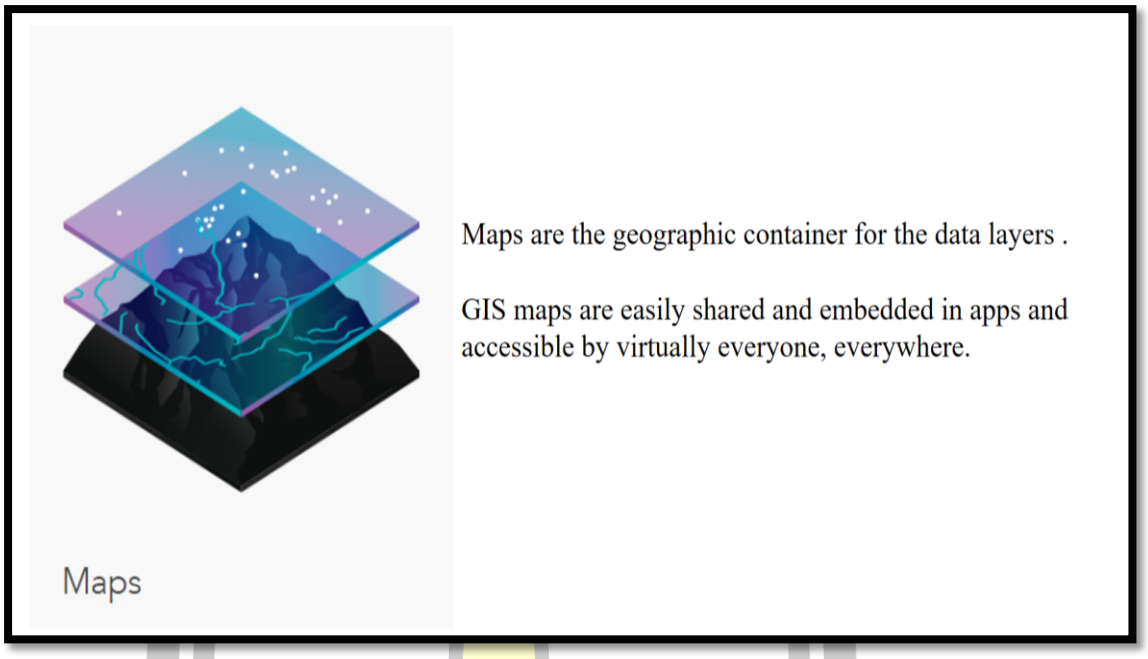


Figure 9 Showing Drive Decisions with Spatial Analysis (Maps)

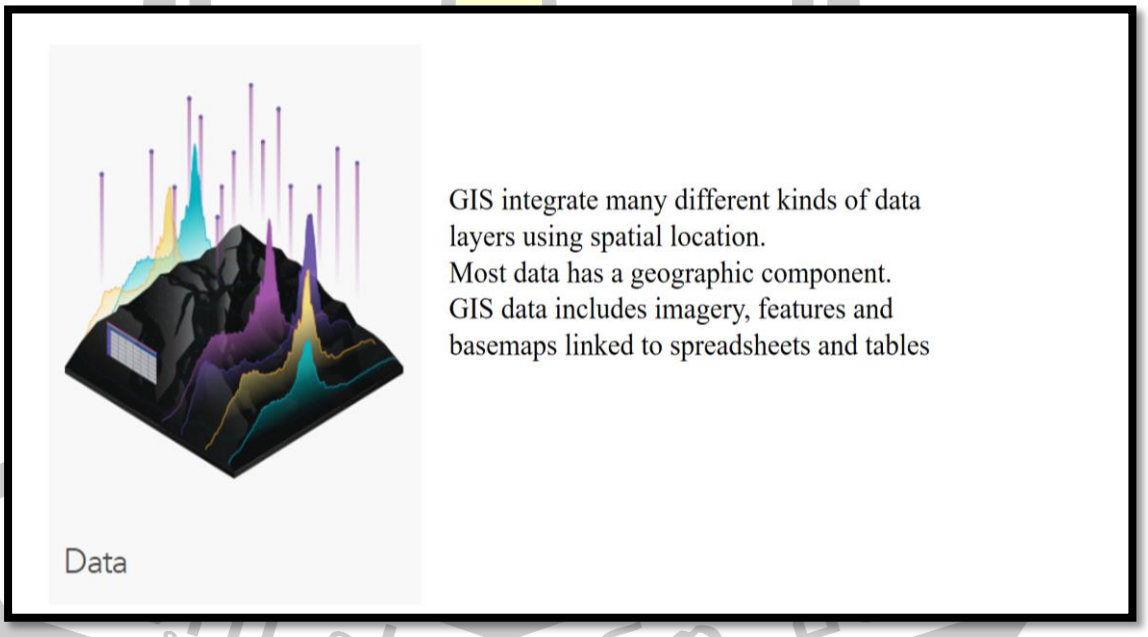


Figure 10 Showing Drive Decisions with Spatial Analysis (Data)

Figure 11 Showing Drive Decisions with Spatial Analysis (Analysis)

Figure 12 Showing Drive Decisions with Spatial Analysis (Apps)

Table 15 Showing the Geographic data of study locations

Location	Lat	Long	Population	Area	Distance from mekong	Height sea level	rain fall
Tachileik	20.4521	99.8989	148,021	1895.8 km²	29 km	395.09m	1570 mm of rain fall per year
Bago	17.3221	96.4663	491,434	7,830 km²	1128 km	4m	3810 mm of rain fall per year
Chom Thong	18.4205	98.6724	66,353	712.3 km²	898.5 km	2,565 m	347 mm of rain fall per year
Mae Ngat	19.1741	99.0307	131,091	3,687 km²	888.9km	310 m	1185 mm of rain fall per year of rainfall per year
Nikhom kham soi	16.3356	104.5597	42,225	377.2 km²	39.7 km	179 m	869 mm of rain fall in a year
Sakon Nakhon	17.1546	104.1348	1,152,282	9,606 km²	120.4 km	175 m	1572 mm of rain fall per year
Sisaket	15.1186	104.322	1,473,011	8,840 km²	211.3 km	133m	986 mm of rain fall in a year
Kalasin	16.4385	103.5061	985,346	6,947 km²	159.1 km	146.91m	1407 mm of rain fall per year

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials and research instruments

1) Blender (Warring: Model 32Bl 80)

2) Binocular compound microscope (Olympus CH-2)

3) Digital cameras (Olympus DP-20) with linked monitor

4) Drawing Tube (camera lucida)

5) Glass wares; Petri dish, beakers, dropper, slides, cover slides, Erlenmeyerflask, cylinder, different sizes bottles and staining jars

6) Hot plate/ slide warmer bench

7) Ocular and stage micrometer

8) Others; tissue paper, pencil/pen, ruler, label pad, brush, aluminum foil and plastic bags

9) Sieve mesh (20, 40, 60 and 80 µm)

10) Stereo microscope (Olympus SZ- 30)

11) Surgical equipment; Surgical blade, scissor, needle, forceps and aluminum/ plastic trays

3.2 Chemical reagents

1) Bouin's Fixative

2) Butyl alcohol

3) Ethyl alcohol 10%,20%, 30% ,50%, 70%, 80% and 95%

4) Formalin 10%

5) Hydrochloric acid (HCL)

6) NaCl 0.85%

7) Permount (Fisher)

8) Pepsin solution 1%

9) Staining agents (Haematoxylin and Borax carmine)

10) Xyline

3.3 Methods

Table 16 Showing the locations that Freshwater fishes collected

Location number	Countries	Freshwater fishes collected Area
1	Myanmar	Bago, Central region of Myanmar
2		Tachileik, Mekong region of Myanmar
3	North of Thailand	Mae Ngat reservoir, Chaing Mai, North of Thailand
4		Chom Thong District, Chaing Mai, North of Thailand
5	Northeast of Thailand	Nikhom Kham Soi, Northeast of Thailand
6		Kalasin, Northeast Thailand
7		Sakon Nakhon, Northeast Thailand
8		Sisaket, Northeast Thailand

3.3.1 Collection of cyprinoid fishes and identification of fish species

Cyprinoid fish were bought from local fish markets.

From Myanmar,

Location -1; Total of 689 fishes (12 different species of cyprinoid fishes) were collected from Tachileik, lower Mekong region of Myanmar (Table 18)

Location -2; Total of 125 fishes (4 different species of cyprinoids fishes) were collected from Bago, Central region of Myanmar (Table 19).

From North of Thailand,

Locaton - 3; Total of 530 fishes (8 different species of cyprinoids fishes) were collected from local market Chiang Mai, North of Thailand (Table 20).

Location -4; Total of 318 fishes (5 different species of cyprinoids fishes) were collected Mae Ngat reservoir, Chiang Mai, North of Thailand (Table 21).

From Northeast of Thailand,

Location-5; Total of 335 fishes (5 different species of cyprinoids fishes) were collected from Nikom KomeSoi, Northeast of Thailand (Table 22).

Location-6; Total of 285 fishes (3different species of cyprinoids fishes) were collected from Sakon Nakhon, Northeast of Thailand (Table 23).

Location-7; Total of 168 fishes (6 different species of cyprinoids fishes) were collected from Sisaket, Northeast, Thailand (Table 24).

Location-8; Total of 434 fishes (5 different species of cyprinoids fishes) were collected from Kalasin, Northeast of Thailand (Table 25).

Table 17 Total Number of Fresh water cyprinoid fishes and species of fishes collected from Myanmar and Thailand

No	Location of fish collection	Latitude	longitude	Total number of Fishes examined	Total Fish Species
1	Tachileik, lower Mekong region of Myanmar	20.4521	99.8989	689	12
2	Bago, Central region of Myanmar.	17.3221	96.4663	125	4
3	Chom Thong District, Chiang Mai, North of Thailand.	18.4205	98.6724	530	8
4	Mae Ngat reservoir, Chiang Mai, North of Thailand	19.1741	99.0307	318	5
5	Nikom KomeSoi, Northeast of Thailand.	16.3356	104.5597	335	5
6	Sakon Nakhon, Northeast of Thailand	17.1546	104.1348	285	3
7	Sisaket,Northeast of Thailand	15.1186	104.322	168	6
8	Kalasin, Northeast of Thailand	16.4385	103.5061	434	5
	Total			2884	48

The cyprinoid fishes were collected during January 2018 to December 2019. All collected fishes were transferred on ice to the laboratory of Tropical and Parasitic Diseases Research Unit, Faculty of Medicine, Mahasarakham University. The length and width of fishes were individually measured and the species of all

fishes were identified with the aid of the fish base website (http://www.fishbase.org/search.php).

Figure 13 Photo of the laboratory of Department of Medicine, Mahasarakhum University

Table 18 Freshwater cyprinoids fishes purchased from the local market in Tachileik, Mekong region of Myanmar

Number	Fish Species	Total number	Average length	Average width
1	*Barbonymus balleroides*	20	9.5cm ± 0.2236	3cm ± 0.3244
2	*Puntioplites falcifer*	30	9.5cm ± 0.2170	3cm ± 0.2110
3	*Systomus rubripinnis*	28	9cm ± 0.3502	3cm ± 0.2377
4	*Labiobarbus siamensis*	60	10cm ± 0.219	3.3cm ± 0.3188
5	*Henicorhynchus siamensis*	50	12.5cm ± 0.2175	3.5cm ± 0.3023
6	*Mystacoleucus marginatus*	100	9.5cm ± 0.2564	3.3cm ± 0.3128
7	*Rasbora argyrotaenia*	20	10.5 cm ± 0.2236	3.5cm ± 0.3244
8	*Systomus orphoides*	10	12.5cm ± 0.9368	3.5cm ± 0.3496

Table 18 (Continued)

Number	Fish Species	Total number	Average length	Average width
9	*Puntius brevis*	100	10.5cm ± 0.2422	3.5cm ± 0.2983
10	*Trichogaste trichopterus*	20	11.5cm ± 0.2236	3.5cm ± 0.2511
11	*Cyclocheilichthys repasson*	151	9.5cm ± 0.2230	3.5cm ± 0.2804
12	*Anabas testudineus*	100	9cm ± 0.2240	3cm ± 0.2876
	total	689		

Table 19 Freshwater cyprinoids fishes purchased from Bago, the Central region of Myanmar

Number	Fish Species	Total number	Average length	Average width
1	*Barbonymus gonionotus*	35	9.5cm ± 0.2170	3cm ± 0.2110
2	*Cyclocheilichthys repasson*	35	9cm ± 0.3502	3cm ± 0.2377
3	*Puntioplites Falcifer*	30	10cm ± 0.219	3.3cm ± 0.3188
4	*Balantiocheilos melanopterus*	25	12.5cm ± 0.2175	3.5cm ± 0.3023
5	Total	125		

Table 20 Freshwater cyprinoids fishes purchased from Chom Thong District, Chiang Mai, North of Thailand.

Number	Fish Species	Total number	Average length	Average width
1	*Thynnichthys thynnoides*	150	9cm ± 0.3502	3cm ± 0.2377
2	*Puntioplites proctozysron*	39	10cm ± 0.219	3.3cm ± 0.3188
3	*Mystacoleucus marginatus*	98	12.5cm ± 0.2175	3.5cm ± 0.3023
4	*Systomus orphoides*	110	9.5cm ± 0.2564	3.3cm ± 0.3128
5	*Barbonymus schwanenfeldii*	93	10.5 cm ± 0.2236	3.5cm ± 0.3244
6	*Osteochilus vittatus*	20	12.5cm ± 0.9368	3.5cm ± 0.3496
7	*Hampala dispa*	10	10.5cm ± 0.2422	3.5cm ± 0.2983
8	*Rasbora tornieri*	10	11.5cm ± 0.2236	3.5cm ± 0.2511
	Total	530		

Table 21 Freshwater cyprinoids fishes purchased from Mae Ngat reservoir, Chiang Mai, North of Thailand

Number	Fish Species	Total number	Average length	Average width
1	*Puntioplites proctozysron*	39	10.5 cm ± 0.2236	3.5cm ± 0.3244
2	*Systomus orphoides*	89	12.5cm ± 0.9368	3.5cm ± 0.3496
3	*Barbonymus schwanenfeldii*	60	10.5cm ± 0.2422	3.5cm ± 0.2983
4	*Osteochilus vittatus*	20	11.5cm ± 0.2236	3.5cm ± 0.2511
5	*Thynnichthys thynnoides*	110	9.5cm ± 0.2230	3.5cm ± 0.2804
	Total	318		

Table 22 Freshwater cyprinoids fishes purchased from Nikom KomeSoi, Northeast of Thailand

Number	Fish Species	Total number	Average length	Average width
1	Barbonymus gonionotus	141	9.5cm ± 0.2564	3.3cm ± 0.3128
2	Systomus rubripinnis	44	10.5 cm ± 0.2236	3.5cm ± 0.3244
3	Balantiocheilos ambusticauda	122	12.5cm ± 0.9368	3.5cm ± 0.3496
4	Hampala Dispar	10	10.5cm ± 0.2422	3.5cm ± 0.2983
5	Barbonymus schwanenfeldii	18	11.5cm ± 0.2236	3.5cm ± 0.2511
	Total	335		

Table 23 Freshwater cyprinoids fishes from Sakon Nakhon, Northeast of Thailand

Number	Fish Species	Total number	Average length	Average width
1	*Osteochilus vittatus*	67	10cm ± 0.219	3.3cm ± 0.3188
2	*Mystacoleucus marginatus*	153	12.5cm ± 0.2175	3.5cm ± 0.3023
3	*Henicorhychus siamemsis*	65	9.5cm ± 0.2564	3.3cm ± 0.3128
4	Total	285		

Table 24 Freshwater cyprinoids fishes Sisaket, Northeast of Thailand

Number	Fish Species	Total number	Average length	Average width
1	*Systomus orphoides*	24	10.5 cm ± 0.2236	3.5cm ± 0.3244
2	*Balantiocheilos ambusticauda*	30	12.5cm ± 0.9368	3.5cm ± 0.3496
3	*Hampala Dispar*	10	10.5cm ± 0.2422	3.5cm ± 0.2983
4	*Hampala macrolepidota*	10	11.5cm ± 0.2236	3.5cm ± 0.2511
5	*Barbonymus schwanenfeldii*	84	9.5cm ± 0.2230	3.5cm ± 0.2804
6	*Puntioplites falcifer*	10	9cm ± 0.2240	3cm ± 0.2876
	Total	168		

Table 25 Freshwater cyprinoids fishes purchased from Kalasin, Northeast of Thailand

Number	Fish Species	Total number	Average length	Average width
1	Cyclocheilichthys repasson	124	12.5cm ± 0.2175	3.5cm ± 0.3023
2	Barbonymus belinka	60	9.5cm ± 0.2564	3.3cm ± 0.3128
3	Hampala Dispar	50	10.5 cm ± 0.2236	3.5cm ± 0.3244
4	Barbonymus gonionotus	10	12.5cm ± 0.9368	3.5cm ± 0.3496
5	Puntioplites falcifer	100	10.5cm ± 0.2422	3.5cm ± 0.2983
	Total	434		

3.3.2 Pepsin-HCl artificial digestion method

Examination of zoonotic trematode metacercariae in the second intermediate hosts was done by pepsin-HCl artificial digestion techniques. All the collected fishes were ground one by one in a mortar with pestle, and then, the ground samples were transferred into a beaker and mixed with artificial gastric juice. The artificial gastric juice is made by a mixture of 10 gm pepsin A, 10 ml of concentrated HCl and 0.85% NaCl or normal saline 1000 mL (Nithikathkul C, 2008). The grounded samples were mixed well and were placed in a 37 ℃ incubator for 2 h with occasional stirring and removed the larger particles by the filtration of digested materials. Then 0.85% saline was added to the digested sample, and we let it stand for a while and discarded the supernatant very carefully and kept the sediment. The

procedures were repeated 8 or 9 times until the supernatant became clear and a small quantity of the sediment was transferred into a Petri dish containing 6–7 ml physiological saline after which metacercariae were observed and identified using a stereomicroscope and light microscope. The detected metacercariae were isolated and were put into a small dish. Finally, the number of metacercariae of each fluke species were counted for further analysis.

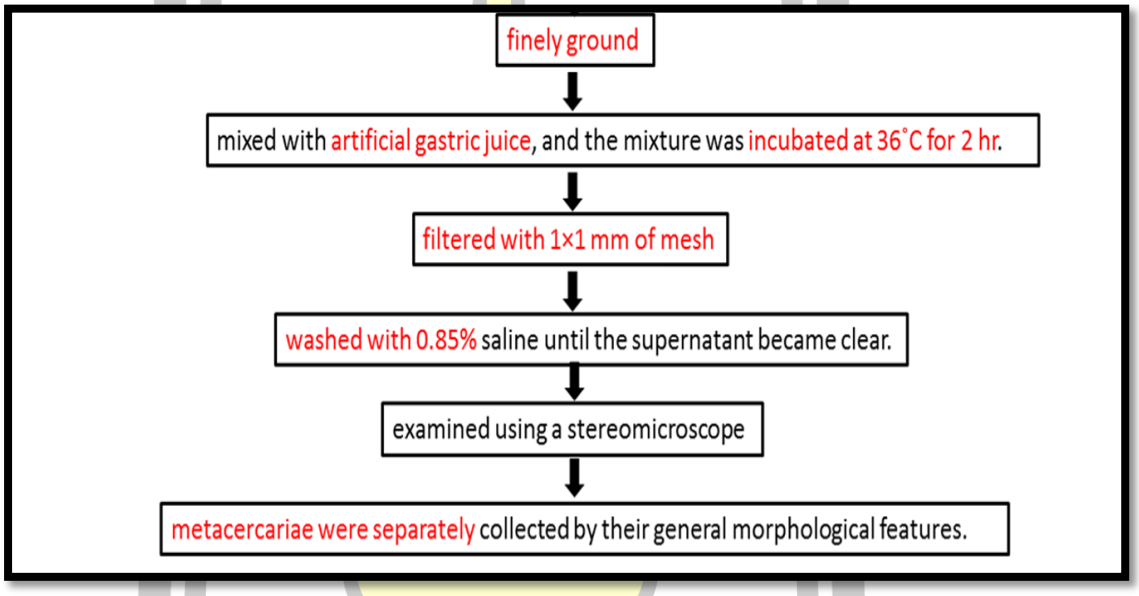


Figure 14 Diagram of the Pepsin Digestion Methods

Figure 15 Photo of Incubator

Figure 16 Photo of the digested sample

3.3.3 Identification of metacercariae

For the identification of metacercariae, first of all, similar-shaped metacercariae were collected separately based on the general morphological features in a small Petri dish. Secondly, they were moved with a spoid onto a glass slide and were covered with a coverslip, then detailed morphology was observed under a light microscope. Finally, the metacercariae were identified based on characteristic morphological features. As the characteristic features, the shape of cysts, size of suckers, shape and contents of the excretory bladder were identified (Sohn, 2009)

3.3.4 Analysis of Findings

After collected metacercariae were categorized according to the size and morphological characteristics, their percentage prevalence was calculated as follows:

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fish} \times 100}{\text{Total number of fish examined}}$$

Intensity is the number of metacererariae per total number of fish infected.

3.4 Geographic Information Systems (GIS) of Fish- borne trematodes Metacercariae

In this study, after fishes were examined for the presence of metacercariae by the digestion technique, Observed results were then summarized as cumulative prevalence for Fish- borne trematodes Metacercariae infections in a tubular format.

To create a GIS database for fish borne trematode infections, each record in the prevalence and intensity tables were associated with geographic coordinates where fishes were taken from using Global Positioning System (GPS) and converted into a shape file with ArcGIS Desktop Program from ESRI Company.

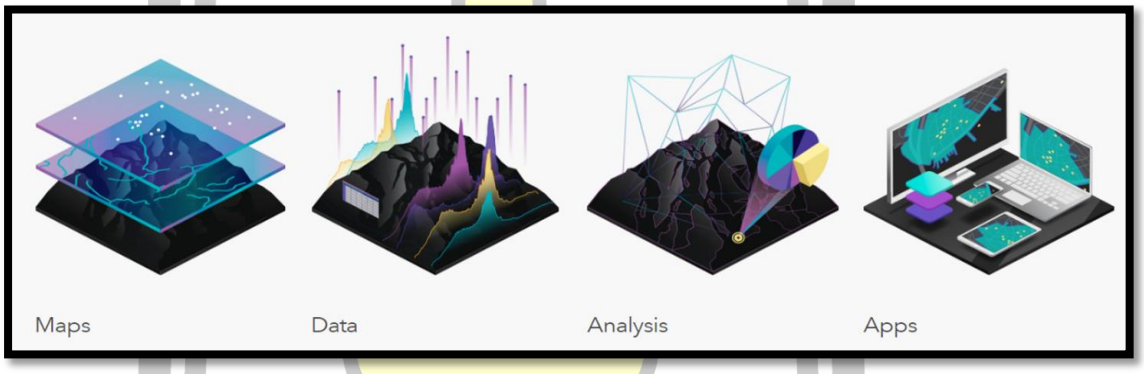


Figure 17 Showing about the Geographic Information analysis

CHAPTER 4

RESULTS

A total of 2884 fishes (48 species) were investigated from total 8 locations from Mekong region of Myanmar, central region of Myanmar, north of Thailand and northeast of Thailand ie; Tachileik, Bago, Chom Thong, Mae Ngat, NikhomKham Soi, Sakon Nakhon, Sisaket, Kalasin.1359 fishes (27 species) were positive with fish borne trematode matacercariae infections. Four Species of fish borne trematode metacercariae i.e.; small liver fluke, *Opisthorchis viverrini*, minute intestinal flukes *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchoides* sp. were detected in this study.

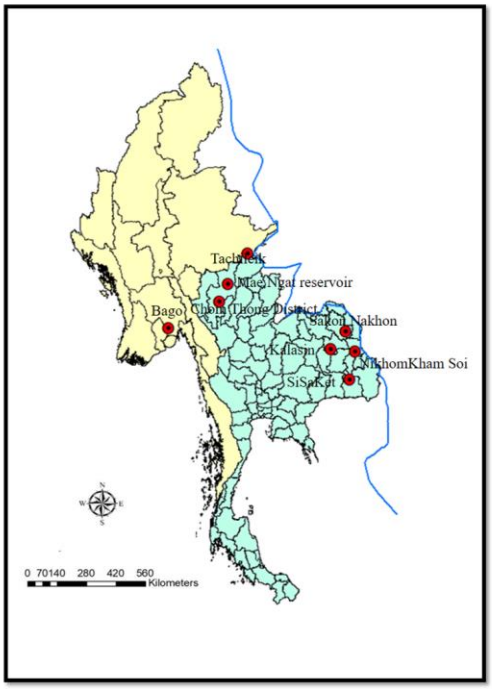


Figure 18 Geographic mapping of freshwater cyprinoid fishes collected area Mekong region of Myanmar, Central region of Myanmar, north of Thailand and northeast of Thailand by ArcGIS 10.5.

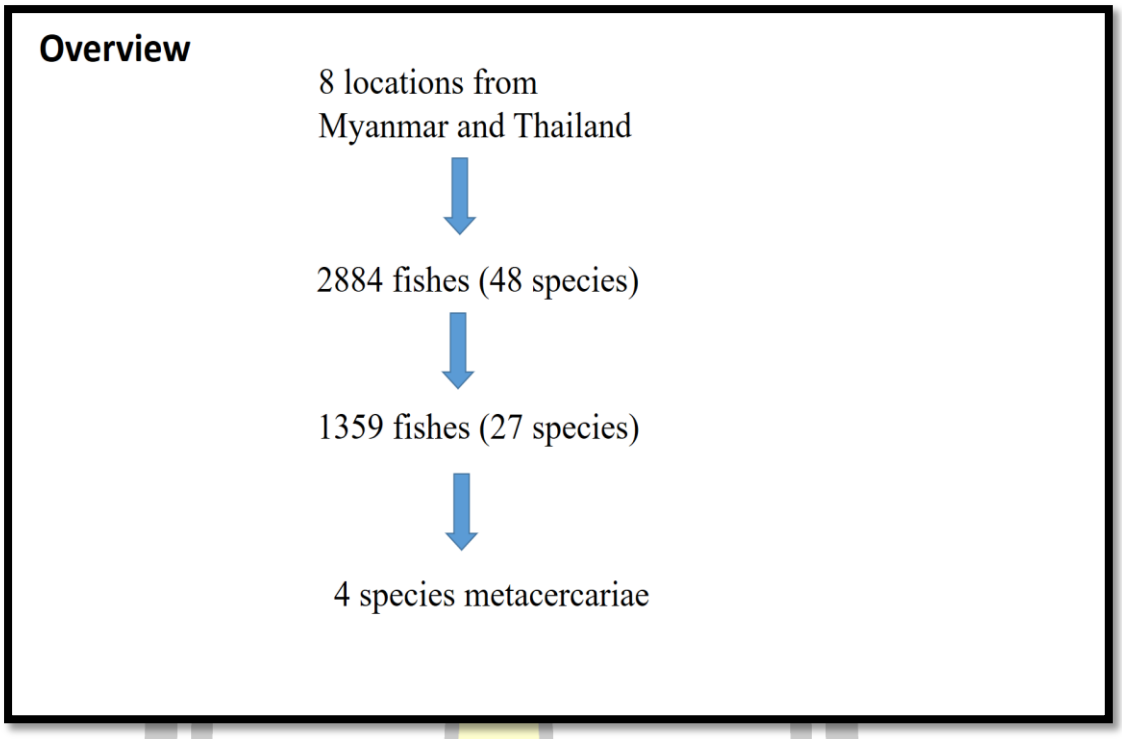


Figure 19 Flow chart of the overview of the research study

Table 26 Total Number of fishes and Prevalence of fish borne trematode infections collected from Myanmar and Thailand

No	Location	Total No of Fishes examined	Total Fish Species	Positive Fish Species	Positive number of Fishes	Negative number of Fishes	Prevalence of FZT
1	Tachileik	689	12	9	319	370	46.29%
2	Bago	125	4	3	56	69	44.80%
3	Chom Thong	530	8	4	284	246	53.58%
4	Mae Ngat	318	5	3	129	189	40.56%
5	Nikhom kham soi	335	5	2	185	150	55.23%
6	Sakon Nakhon	285	3	2	165	120	57.89%
7	Sisaket	168	6	1	46	122	27.38%
8	Kalasin	434	5	3	175	259	40.32%
	Total	2884	48	27	1359	1525	47.12%

4.1 Occurrence of fish borne trematode infections in freshwater fishes from Tachileik, Mekong region of Myanmar

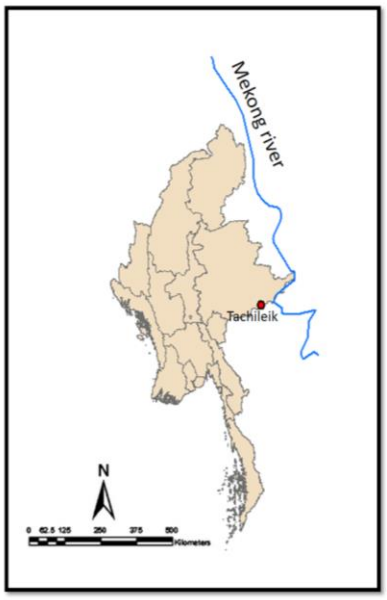


Figure 20 Geographic mapping of freshwater cyprinoid fishes collected area from Tachileik lower Mekong region of Myanmar (20°27′N 99°53′E) by ArcGIS 10.5.

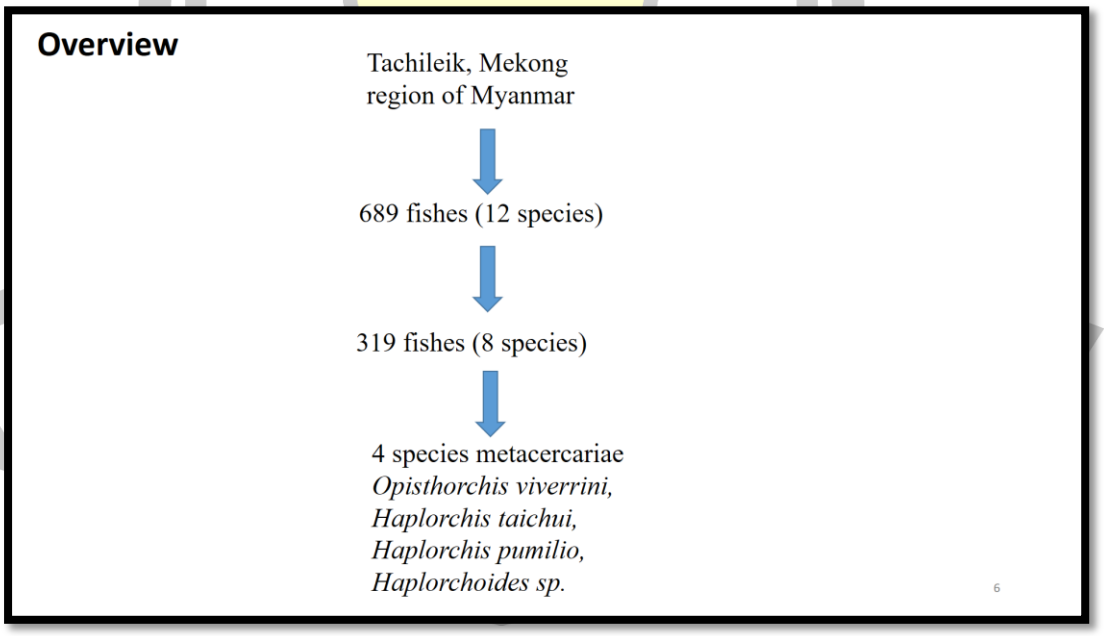
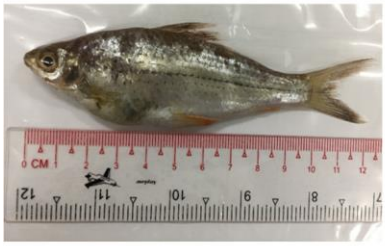


Figure 21 Flow chart of the overview of the research study from Tachileik lower Mekong region of Myanmar

Barbonymus balleroides

Puntioplites Falcifer

Systomus rubripinnis



labiobarbus siamensis

henicorhynchus siamensis

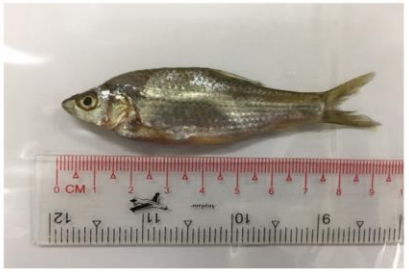
mystacoleucus marginatus

rasbora argyrotaenia

Systomus orphoides

Puntius brevis

Trichogaster trichopterus



Cyclocheilichthys repasson

Anabas testudineus

Figure 22 Photos of fresh water cyprinoid fishes collected from Tachileik, Mekong region of Myanmar

4.1.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Tachileik, Mekong Region of Myanmar

In the total of 689 fishes, 319 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 46.29%. Among the 12 different species, 8 species of cyprinoid fishes, i.e., *B. gonionotus*, *P. falcifer*, *M. marginatus*, *C. repasson*, *S. Rubripinnis*, *l. siamensis*, *H. siamensis*, *R. argyrotaenia*, were contaminated with the infective stage of trematode parasites, metacercariae. The metacercariae of *O. viverrini* were found in *C. repasson* (2.64%, *n* = 151). The metacercariae of *H. taichui* were found infecting 4 species among the total 12 species of fishes. The highest infection prevalence among the four species of fish was found in *R. argyrotaenia* (45%, *n* = 20), and then, in decreasing order, in *M. marginatus* (38%, *n* = 100), *C. repasson* (35%, *n* = 151) and *L. siamensis* (20%, *n* = 60). The metacercariae of *H. pumilio* were infected in 5 out of 12 species of fishes. The highest infection prevalence among the four species of fishes was found in *B. balleroides* (65%, *n* = 20) and then with decreasing order in *Systomus rubripinnis* (60.71%, *n* = 28), *P. falcifer* (53.33%, *n* = 30), *M. marginatus* (14%, *n* = 100) and *C. repasson* (7.94%, *n* = 151). The metacercariae *Haplorchoides* sp. were present in 5 out of 12 species of fishes. The highest prevalence was detected in *C. repasson* (82.78%, *n* = 151), and the lowest prevalence was occurred in *H. siamensis* (56%, *n* = 50) and the others were found in *L. siamensis* (63.33%, *n* = 60), *M. marginatus* (68%, *n* = 100) and *R. argyrotaenia* (70%, *n* = 20), respectively.

4.1.2 Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Tachileik, Mekong Region of Myanmar

Simultaneous infections of one fish with two or more trematode species were recorded in some fish species, i.e., *L. siamensis*, *M. marginatus*, *R. argyrotaenia*, *C. repasson. Four* species of fish-borne trematode metacercariae, i.e., *O. viverrini*, *H. taichui*, *H. pumilio*, *Haplorchoides* sp. simultaneously infected *C. repasson.* The fish species *M. marginatus* was infected with *H. taichui*, *H. pumilio*, *Haplorchoides* sp. Co-infection of trematode metacercariae *H. taichui*, *Haplorchoides* sp. was observed in *L. siamensis* and *R. argyrotaenia.*

Table 27 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Tachileik, Mekong region of Myanmar

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Barbonymus balleroides*	20	13 (65)	_	_	13 (65)	_
2	*Puntioplites falcifer*	30	16 (53.33)	_	_	16 (53.33)	_
3	*Systomus rubripinnis*	28	17 (60.71)	_	_	17 (60.71)	_
4	*Labiobarbus siamensis*	60	38 (63.33)	_	12 (20)	_	38 (63.33)
5	*Henicorhynchus siamensis*	50	28 (56)	_	_	_	28 (56)
6	*Mystacoleucus marginatus*	100	68 (68)	_	38 (38)	14 (14)	68 (68)
7	*Rasbora argyrotaenia*	20	14 (70)	_	9 (45)	_	14 (70)
8	*Systomus orphoides*	10	0 (0)	_	_	_	_
9	*Puntius brevis*	100	0 (0)	_	_	_	_
10	*Trichogaster trichopterus*	20	0 (0)	_	_	_	_
11	*Cyclocheilichthys repasson*	151	125 (82.78)	4 (2.64)	35 (23.17)	12 (7.94)	125 (82.78)
12	*Anabas testudineus*	100	0 (0)	_	_	_	_
	Total	689	319 (46.29)				

4.1.3 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Tachileik, Mekong Region of Myanmar

A total of 11 metacercariae of *O. viverrini* were detected from *C. repasson* with a mean intensity of 2.75 per fish infected. The metacercariae of *H. taichui* were collected in 94 (28.39%) out of 331 fishes (4 species), i.e., *L. siamensis, M. marginatus, R. argyrotaenia, C. repasson*, with a mean intensity of 3.75 per fish infected. The metacercariae of *H. pumilio* were detected in 72 (21.88%) out of 329 fishes (5 species), i.e., *B. gonionotus, P. falcifer, S. rubripinnis, M. marginatus, C. repasson*, with a mean intensity of 4.11 per fish infected. The metacercariae of *Haplorchoides* sp. were detected in 273 (71.65%) out of 381 fishes (5 species), i.e., *L. siamensis, H. siamensis, M. marginatus, R. argyrotaenia, C. repasson*, with a mean intensity of 3.57 per fish infected.

Table 28 Intensity of *O. viverrini* metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

No	Fish Species	No. of fish examined	No. (%) of fish infected OV	total metacercariae detected	Range (Min-Max)	Intensity
1	*Cyclocheilichthys repasson*	151	4 (2.64)	11	(2-3)	2.75

Ov, *O. viverrini*.

Table 29 Intensity of *H. taichui* metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

No	Fish Species	No. of fish examined	No. (%) of fish infected HT	total metacercariae detected	Range (Min-Max)	Intensity
1	*Labiobarbus siamensis*	60	12 (20)	102	(3-12)	8.5
2	*Mystacoleucus marginatus*	100	38 (38)	120	(2-10)	3.15
3	*Rasbora argyrotaenia*	20	9 (45)	10	(1-2)	1.11
4	*Cyclocheilichthys repasson*	151	35 (23.17)	121	(1-9)	3.45
	Total	331	94 (28.39)	353	(1-12)	3.75

Ht, *H. taichui*.

Table 30 Intensity of *H. pumilio* metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar

No	Fish Species	No. of fish examined	No. (%) of fish infected	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus balleroides*	20	13 (65)	24	(1-4)	1.84
2	*Puntioplites falcifer*	30	16 (53.33)	16	(1-1)	1
3	*Systomus rubripinnis*	28	17 (60.71)	59	(1-7)	3.47
4	*Mystacoleucus marginatus*	100	14 (14)	67	(1-6)	4.78

Table 30 (Continued)

No	Fish Species	No. of fish examined	No. (%) of fish infected	total metacercariae detected	Range (Min-Max)	Intensity
5	*Cyclocheilichthys repasson*	151	12(7.94)	130	(1-21)	10.83
	Total	329	72(21.88)	296	(1-21)	4.11

Hp, *Haplorchis pumilio*.

Table 31 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Labiobarbus siamensis*	60	38 (63.33)	102	(1-7)	2.68
2	*Henicorhynchus siamensis*	50	28 (56)	43	(1-3)	1.53
3	*Mystacoleucus marginatus*	100	68 (68)	120	(1-5)	1.76
4	*Rasbora argyrotaenia*	20	14 (70)	16	(1-2)	1.14
5	*Cyclocheilichthys repasson*	151	125 (82.78)	695	(1-21)	5.56
	Total	381	273 (71.65)	976	(1-21)	3.57

Hap, *Haplorchoides* sp.

4.2 Occurrence of fish borne trematode infections in freshwater fishes from Bago, Central region of Myanmar.

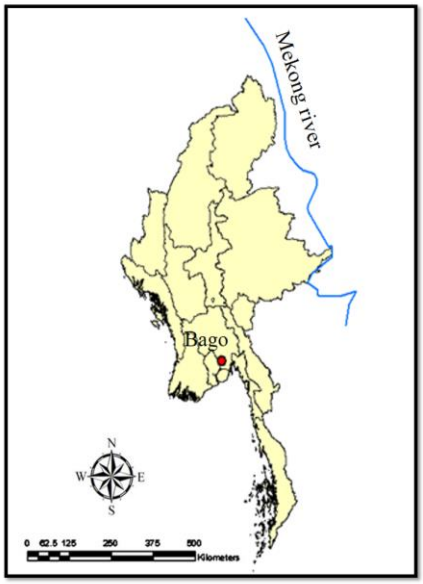


Figure 23 Geographic mapping of freshwater cyprinoid fishes collected area from Bago, the Central region of Myanmar. (17°33′N 96°46′E) by ArcGIS 10.5.

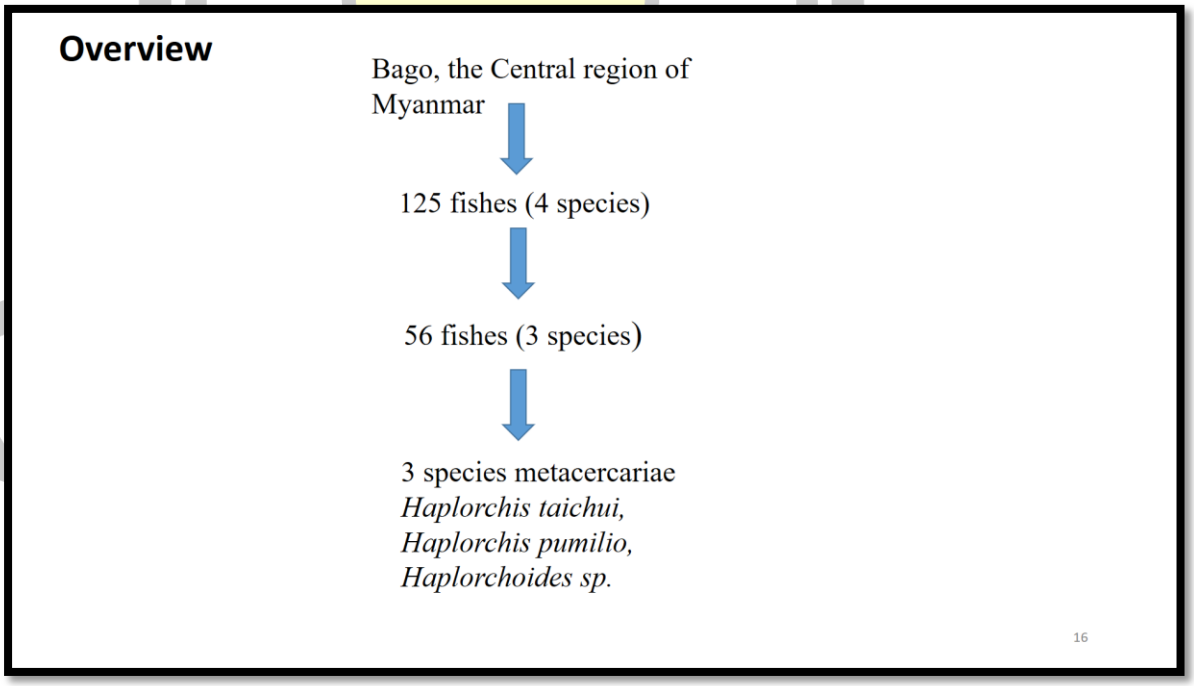
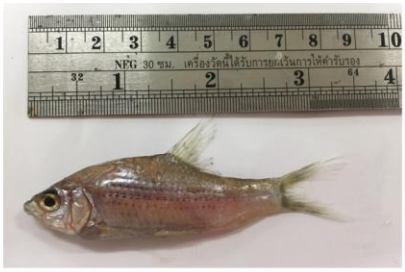
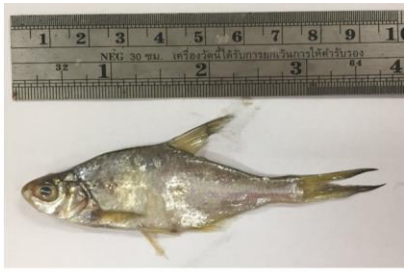


Figure 24 Flow chart of the overview of the research study from Bago, the Central region of Myanmar. (17°33′N 96°46′E) by ArcGIS 10.5.

Barbonymus gonionotus



Cyclocheilichthys repasson



Puntioplites Falcifer

Balantiocheilos melanopterus

Figure 25 Photos of fresh water cyprinoid fishes collected from Bago, the Central region of Myanmar

4.2.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Bago, the Central region of Myanmar

In the total of 125 fishes, 56 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 44.8 %. Among the 4 different species, 3 species of cyprinoid fishes, i.e., *B. gonionotus*, *C. repasson*, *B. melanopterus* were contaminated with the infective stage of trematode parasites, metacercariae. The metacercariae of *H. taichui* were found infecting 3 species among the total 4 species of fishes. The highest infection prevalence among the four species of fish was found in *B. gonionotus* (57.14 %, *n* = 35), and then, in decreasing order, in *B. melanopterus* (48 %, *n* = 25) and *C. repasson* (42,85 %, *n* = 35). The metacercariae of *H. pumilio* were infected in *B. melanopterus* (65%, *n* = 20). The metacercariae *Haplorchoides* sp. were present in 2out of 4 species of fishes. The highest prevalence was detected in *B. gonionotus* (74.28%, *n* = 35), and the lowest prevalence was occurred in *C. repasson* (51.42%, *n* = 35) respectively.

4.2.2 Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Bago, the Central region of Myanmar

Simultaneous infections of one fish with two or more trematode species were recorded in some fish species, i.e., *C. repasson, B. gonionotus, B. melanopterus*. The fish species *C. repasson, B. gonionotus* was infected with *H. taichui, Haplorchoides* sp. Co-infection of trematode metacercariae *H. taichui, H. pumilio* was observed in *B. melanopterus*.

Table 32 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Bago, the Central region of Myanmar

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Barbonymus gonionotus*	35	26 (74.28)	_	20(57.14)	_	26 (74.28)
2	*Cyclocheilichthys repasson*	35	18(51.42)	_	15(42.85)	_	18(51.42)
3	*Puntioplites Falcifer*	30	0(0)	_	_	_	_
4	*Balantiocheilos melanopterus*	25	12(48)	_	12(48)	5 (20)	_
	Total	125	56(44.8)				

4.2.3 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Bago, the Central region of Myanmar

The metacercariae of *H. taichui* were collected in 47 (49.47%) out of 95 fishes (3 species), i.e., *B. gonionotus, B. melanopterus, C. repasson,* with a mean intensity of 2.74 per fish infected (Table 4). The metacercariae of *H. pumilio* were detected in *B. melanopterus* 5 (20 %) with a mean intensity of 4.6 per fish infected. The metacercariae of *Haplorchoides* sp. were detected in 44 (62.85%) out of 164 fishes (2 species), i.e., *B. gonionotus, C. repasson,* with a mean intensity of 3.72 per fish infected (Table 33).

Table 33 Intensity of *H. taichui* metacercariae detected in freshwater fishes from Bago, the Central region of Myanmar

No	Fish Species	No. of fish examined	No. (%) of fish infected HT	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus gonionotus*	35	20 (57.14)	48	1-6	2.4
2	*Cyclocheilichthys repasson*	35	15 (42.85)	35	1-4	2.33
3	*Balantiocheilos melanopterus*	25	12 (48)	46	1-5	3.83
4	Total	95	47(49.47)	129	1-6	2.74

Ht, *H. taichui*.

Table 34 Intensity of *H. pumilio* metacercariae detected in freshwater fishes from Bago, the Central region of Myanmar

No	Fish Species	No. of fish examined	No. (%) of fish infected HP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Balantiocheilos melanopterus*	25	5 (20)	23	1-5	4.6

Hp, *Haplorchis pumilio*.

Table 35 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Bago, the Central region of Myanmar

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus gonionotus*	35	26 (74.28)	95	1-5	3.6
2	*Cyclocheilichthys repasson*	35	18(51.42)	69	1-6	3.83
3	Total	70	44(62.85)	164	1-6	3.72

Hap, *Haplorchoides* sp.

4.3 Occurrence of fish borne trematode infections in freshwater fishes from Chom Thong District, Chiang Mai, Thailand

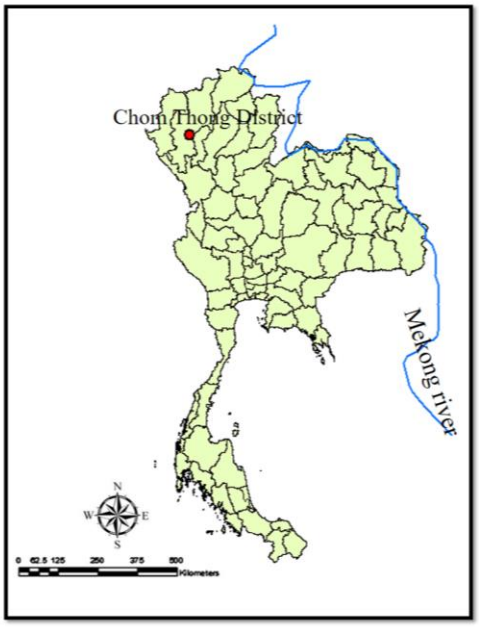


Figure 26 Geographic mapping of freshwater cyprinoid fishes collected area from Chom Thong District, Chiang Mai, Thailand (18°79′N 98°96′E).

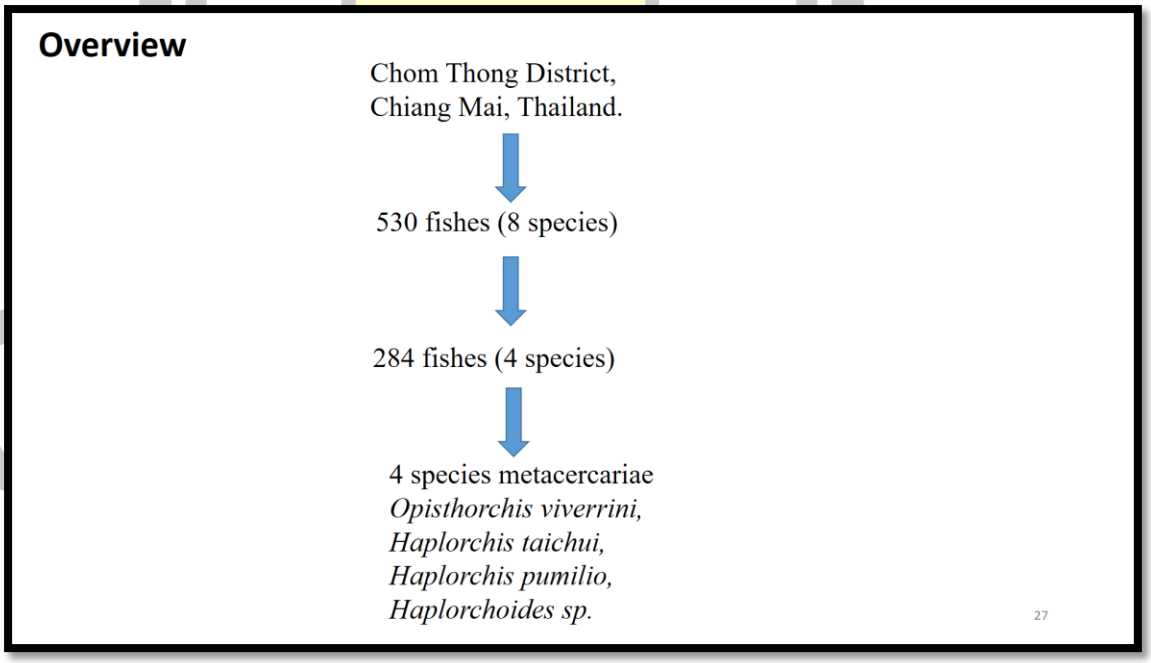


Figure 27 Flow chart of the overview of the research study from Chom Thong District, Chiang Mai, Thailand.

Figure 28 Photos of fresh water cyprinoid fishes collected from Chom Thong District, Chiang Mai, Thailand.

4.3.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Chom Thong District, Chiang Mai, North of Thailand.

In the total of 530 fishes, 284 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 53.58%. Among the 8 different species, 4 species of cyprinoid fishes, i.e., *T. thynnoides, M. marginatus, S. orphoides* and *B. Schwanenfeldii* were contaminated with the infective stage of trematode parasites, metacercariae (Table 36). The metacercariae of *O. viverrini* were found in *Mystacoleucus marginatus* (10.20 %, *n* = 98). The metacercariae of *H. taichui* were

found infecting 4 species among thetotal 8 species of fishes. The highest infection prevalence among the four species of fish was found in *B. schwanenfeldii* (68.51 %, *n* = 93), and then, in decreasing order, in S. *orphoides* (50.90 %, *n* = 110), M. *marginatus* (48.97 %, *n* = 98) and *T. thynnoides* (44.66%, *n* = 150). The metacercariae of *H. pumilio* were infected in 5 out of 12 species of fishes. The highest infection prevalence among the four species of fishes was found in *B. schwanenfeldii* (11.82 %, *n* = 93) and then with decreasing order in *S. orphoides* (60.71%, *n* = 28). The metacercariae *Haplorchoides* sp. were present in 4 out of 8 species of fishes. The highest prevalence was detected in *Thynnichthys thynnoides* (65.33 %, *n* = 150), and the lowest prevalence was occurred in *Barbonymus schwanenfeldii (*58.06%, *n* = 93) and the others were found in *M. marginatus* (65.30%, *n* = 98) and *S. orphoides* (68%, *n* = 100) respectively.

4.3.2 Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Chom Thong District, Chiang Mai, North of Thailand.

Simultaneous infections of one fish with two or more trematode species were recorded in some fish species, i.e., *Mystacoleucus marginatus, Systomus orphoides, Barbonymus schwanenfeldii and Thynnichthys thynnoides* (Table 36). Three species of fish-borne trematode metacercariae, i.e., *O. viverrini, H. taichui, Haplorchoides* sp. simultaneously infected *Mystacoleucus marginatus* (Table 36). The fish species *Systomus orphoides and Barbonymus schwanenfeldii* was infected with *H. taichui, H. pumilio, Haplorchoides* sp. (Table 36). Co-infection of trematode metacercariae *H. taichui, Haplorchoides* sp. was observed in *Thynnichthys thynnoides* (Table 36).

Table 36 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Chom Thong District, Chiang Mai, North of Thailand.

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Thynnichthys thynnoides*	150	98 (65.33)	_	67 (44.66)	_	98 (65.33)
2	*Puntioplites proctozysron*	39	0 (0)	_	_	_	_
3	*Mystacoleucus marginatus*	98	64 (65.30)	10 (10.20)	48 (48.97)	_	64 (65.30)
4	*Systomus orphoides*	110	68 (61.18)	_	56 (50.90)	8 (7.27)	68 (61.18)
5	*Barbonymus schwanenfeldii*	93	54 (58.06)	_	37 (68.51)	11 (11.82)	54 (58.06)
6	*Osteochilus vittatus*	20	0 (0)	_	_	_	_
7	*Hampala dispa*	10	0 (0)	_	_	_	_
8	*Rasbora tornieri*	10	0 (0)	_	_	_	_
	Total	530	284 (53.58)				

4.3.3 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Chom Thong District, Chiang Mai, North of Thailand.

A total of 10 metacercariae of *O. viverrini* were detected from *Mystacoleucus marginatus* with a mean intensity of 2.75 per fish infected (Table 37). The metacercariae of *H. taichui* were collected in 208 (46.11%) out of 451 fishes (4 species), i.e., *Thynnichthys thynnoides, Mystacoleucus marginatus, Systomus orphoides, Barbonymus schwanenfeldii,* with a mean intensity of 1.37 per fish infected (Table 38). The metacercariae of *H. pumilio* were detected in 19 (9.3%) out of 203 fishes (2 species), i.e., *Systomus orphoides, Barbonymus schwanenfeldii* with a mean intensity of 4.72 per fish infected (Table 39). The metacercariae of *Haplorchoides* sp. were detected in 284 (62.97%) out of 451 fishes (4 species), i.e., *Thynnichthys thynnoides, Mystacoleucus marginatus, Systomus orphoides, Barbonymus schwanenfeldii* with a mean intensity of 6.86 per fish infected (Table 40).

Table 37 Intensity of *O. viverrini* metacercariae detected in freshwater fishes from Chom Thong District, Chiang Mai, North of Thailand.

No	Fish Species	No. of fish examined	No. (%) of fish infected OV	total metacercariae detected	Range (Min-Max)	Intensity
1	*Mystacoleucus marginatus*	98	10 (10.20)	10	1	1

Ov, *O. viverrini*.

Table 38 Intensity of *H. taichui* metacercariae detected in freshwater fishes from Chom Thong District, Chiang Mai, North of Thailand.

No	Fish Species	No. of fish examined	No. (%) of fish infected HT	total metacercariae detected	Range (Min-Max)	Intensity
1	*Thynnichthys thynnoides*	150	67 (44.66)	102	1-14	1.52
2	*Mystacoleucus marginatus*	98	48 (48.97)	68	1-8	1.41
3	*Systomus orphoides*	110	56 (50.90)	63	1-6	1.12
4	*Barbonymus schwanenfeldii*	93	37 (68.51)	54	1-5	1.45
	total	451	208(46.11)	287	1-14	1.37

Ht, *H. taichui*.

Table 39 Intensity of *H. pumilio* metacercariae detected in freshwater fishes from Chom Thong District, Chiang Mai, North of Thailand.

No	Fish Species	No. of fish examined	No. (%) of fish infected HP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Systomus orphoides*	110	8 (7.27)	29	1-5	3.62
2	*Barbonymus schwanenfeldii*	93	11 (11.82)	56	1-6	5.09
	Total	203	19(9.3)	85	1-6	4.72

Hp, *Haplorchis pumilio*.

Table 40 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Chom Thong District, Chiang Mai, North of Thailand.

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Thynnichthys thynnoides*	150	98 (65.33)	369	1-26	3.76
2	*Mystacoleucus marginatus*	98	64 (65.30)	183	1-18	2.85
3	*Systomus orphoides*	110	68 (61.18)	1337	1-29	19.66
4	*Barbonymus schwanenfeldii*	93	54 (58.06)	61	1-5	1.12
	total	451	284 (62.97)	1950	1-29	6.86

Hap, *Haplorchoides* sp.

4.4 Occurrence of fish borne trematode infections in freshwater fishes from Mae Ngat reservoir, Chiang Mai, North of Thailand.

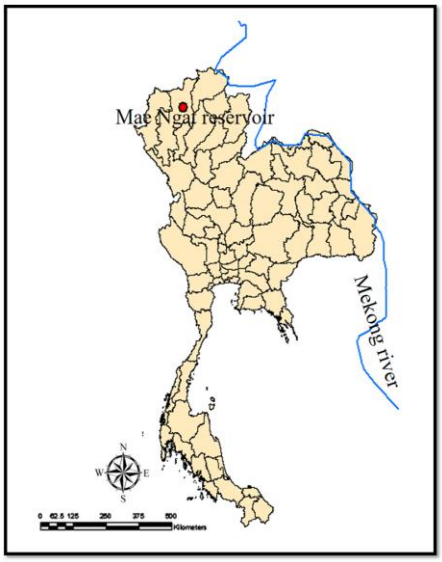


Figure 29 Geographic mapping of freshwater cyprinoid fishes collected area from Mae Ngat reservoir, Chiang Mai, North of Thailand. (19°94′N 99°22′E).

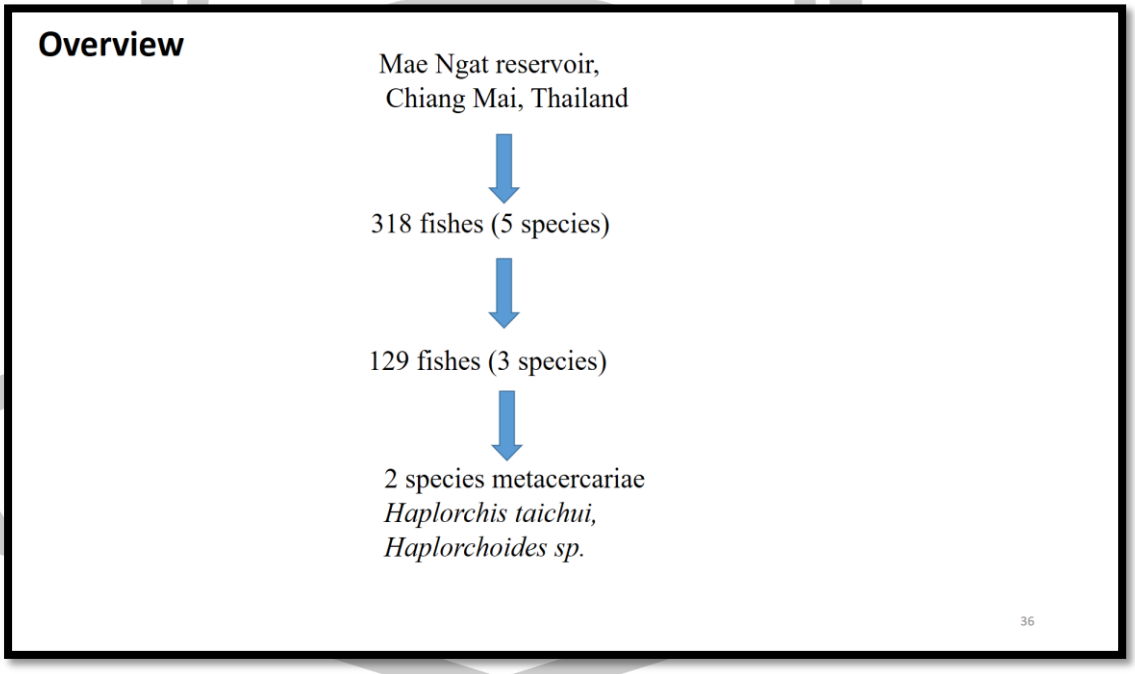


Figure 30 Flow chart of the overview of the research study from Mae Ngat reservoir, Chiang Mai, North of Thailand.

Puntioplites proctozysron

Systomus orphoides

Barbonymus schwanenfeldii

Osteochilus vittatus

Thynnichthys thynnoides

Figure 31 Photos of fresh water cyprinoid fishes collected from Mae Ngat reservoir, Chiang Mai, North of Thailand.

4.4.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Mae Ngat reservoir, Chiang Mai, North of Thailand

In the total of 318 fishes, 129 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 40.56 %. Among the 5 different species, 3 species of cyprinoid fishes, i.e., *Systomus orphoides, Barbonymus schwanenfeldii, Thynnichthys thynnoides* were contaminated with the infective stage of trematode parasites, metacercariae (Table 41). The metacercariae of *H. taichui* were found infecting 2 species among the total 5 species of fishes. The infection prevalence of fish was found in *Barbonymus schwanenfeldii* (26.66%, *n* = 60) and *Thynnichthys thynnoides* (26.36%, *n* = 110). The metacercariae *Haplorchoides* sp. were present in 3 out of 5 species of fishes. The highest prevalence was detected in *Systomus orphoides* (53.39 %, *n* = 89), and the lowest prevalence was occurred in *Thynnichthys thynnoides* (47.27 %, *n* = 110) and the others were found in *Barbonymus schwanenfeldii* (48.33%, *n* = 60), respectively.

4.4.2 Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Mae Ngat reservoir, Chiang Mai, North of Thailand

Simultaneous infections trematode species were recorded in some fish species, i.e., *Barbonymus schwanenfeldii, Thynnichthys thynnoides* (Table 41). Co-infection of trematode metacercariae *H. taichui, Haplorchoides* sp. was observed in *Barbonymus schwanenfeldii, Thynnichthys thynnoides* (Table 41).

Table 41 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Mae Ngat reservoir, Chiang Mai, North of Thailand

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Puntioplites proctozysron*	39	0(0)	_	_	_	_
2	*Systomus orphoides*	89	48(53.39)	_		_	48(53.39)
3	*Barbonymus schwanenfeldii*	60	29(48.33)	_	16(26.66)	_	29(48.33)
4	*Osteochilus vittatus*	20	0(0)	_	_	_	_
5	*Thynnichthys thynnoides*	110	52(47.27)	_	29(26.36)	_	52(47.27)
6	Total	318	129(40.56)				

4.4.3 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Mae Ngat reservoir, Chiang Mai, North of Thailand

The metacercariae of *H. taichui* were collected in 45 (26.47 %) out of 170 fishes (4 species), i.e., *Barbonymus schwanenfeldii*, *Thynnichthys thynnoides* with a mean intensity of 1.97 per fish infected (Table 42). The metacercariae of *Haplorchoides* sp. were detected in 129 (49.80 %) out of 259 fishes (4 species), i.e., *Systomus orphoides*, *Barbonymus schwanenfeldii*, *Thynnichthys thynnoides* with a mean intensity of 1.32 per fish infected (Table 43).

Table 42 Intensity of *H. taichui* metacercariae detected in freshwater fishes from Mae Ngat reservoir, Chiang Mai, North of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HT	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus schwanenfeldii*	60	16(26.66)	31	1-7	1.93
2	*Thynnichthys thynnoides*	110	29(26.36)	58	1-5	2
3	*Total*	170	45(26.47)	89	1-7	1.97

Ht, *H. taichui*.

Table 43 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Mae Ngat reservoir, Chiang Mai, North of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Systomus orphoides*	89	48 (53.39)	87	1-8	1.81
2	*Barbonymus schwanenfeldii*	60	29 (48.33)	82	1-12	2.83
3	*Thynnichthys thynnoides*	110	52 (47.27)	172	1-15	3.31
	Total	259	129 (49.80)	341	1-15	1.32

Hap, *Haplorchoides* sp.

4.5 Occurrence of fish borne trematode infections in freshwater fishes from Nikhom Kham Soi, Northeast of Thailand

Figure 32 Geographic mapping of freshwater cyprinoid fishes collected area from Nikhom Kham Soi, Northeast of Thailand (16°34′N 104°56′E) by ArcGIS 10.5.

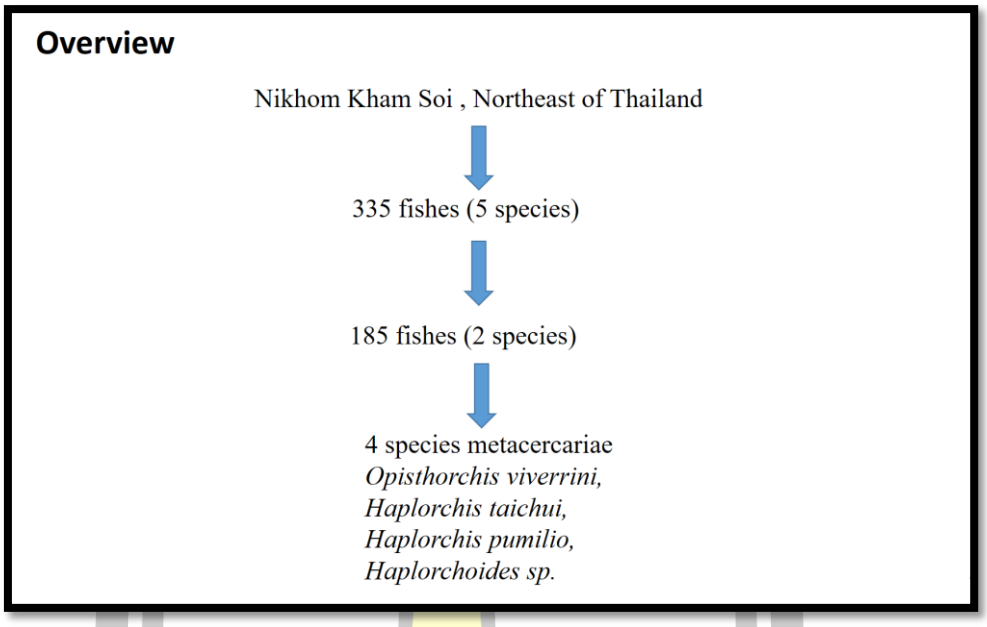


Figure 33 Flow chart of the overview of the research study from Nikhom Kham Soi, Northeast of Thailand

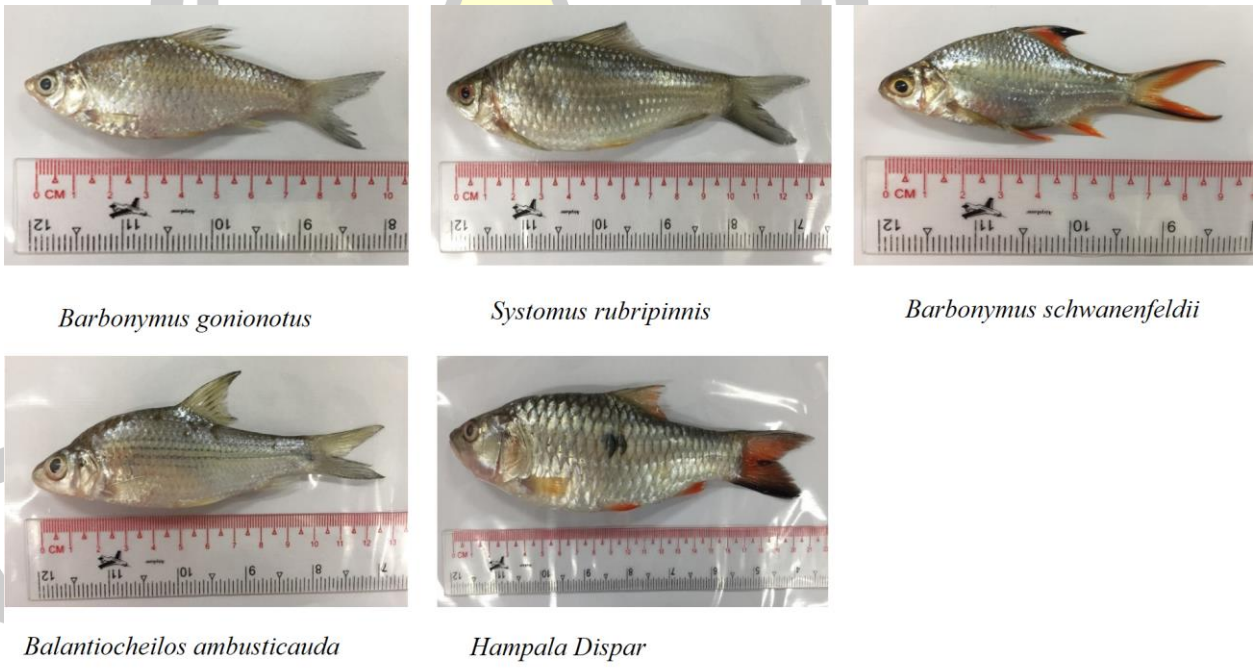


Figure 34 Photos of fresh water cyprinoid fishes collected from Nikhom Kham Soi, Northeast of Thailand

Table 44 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Nikhom Kham Soi, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Barbonymus gonionotus*	141	103(73.04)	_	58(41.13)	_	103(73.04)
2	*Systomus rubripinnis*	44	0(0)	_	_	_	_
3	*Balantiocheilos ambusticauda*	122	82(67.21)	9(7.37)	_	8(6.55)	82(67.21)
4	*Hampala Dispar*	10	0(0)	_	_	_	_
5	*Barbonymus schwanenfeldii*	18	0(0)	_	_	_	_
	Total	335	185(55.22)				

4.5.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Nikhom Kham Soi, Northeast of Thailand

In the total of 335 fishes, 185 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 55.22 %. Among the 5 different species, 2 species of cyprinoid fishes, i.e., *Barbonymus gonionotus*, *Balantiocheilos ambusticauda* were contaminated with the infective stage of trematode parasites, metacercariae (Table 2). The metacercariae of *O. viverrini* were found in *Balantiocheilos ambusticauda* (7.37%, *n* = 122). The metacercariae of *H. taichui* were found in *Barbonymus gonionotus* (41.13 %, n = 141). The metacercariae of *H. pumilio* were infected in *Balantiocheilos ambusticauda* (6.55 %, *n* = 122). The metacercariae *Haplorchoides* sp. were present in 2 out of 5 species of fishes, *Barbonymus gonionotus* (41.13 %, n = 141) and *Balantiocheilos ambusticauda* (6.55 %, n = 122) respectively.

4.5.2 Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Nikhom Kham Soi, Northeast of Thailand

Simultaneous infections of one fish with two or more trematode species were recorded in some fish species, i.e., *Barbonymus gonionotus* and *Balantiocheilos ambusticauda* (Table 44). Three species of fish-borne trematode metacercariae, i.e., *O. viverrini, H. pumilio, Haplorchoides* sp. simultaneously infected *Balantiocheilos ambusticauda* (Table 44). Co-infection of trematode metacercariae *H. taichui, Haplorchoides* sp. was observed in *Barbonymus gonionotus* (Table 44).

4.5.3 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Nikhom Kham Soi, Northeast of Thailand

A total of 15 metacercariae of *O. viverrini* were detected from *Balantiocheilos ambusticauda with* a mean intensity of 1.66 per fish infected (Table 45). The metacercariae of *H. taichui* were collected in 58 (41.13%) out of 141 fishes in *Barbonymus gonionotus* species of fish with a mean intensity of 3.75 per fish infected (Table 46). The metacercariae of *H. pumilio* were detected in 8 (6.55%) out of 122in *Balantiocheilos ambusticauda* species of fishes with a mean intensity of 1.37 per fish infected (Table 47). The metacercariae of *Haplorchoides* sp. were detected in 185 (70.34%) out of 265 fishes (2 species), i.e., *Barbonymus gonionotus, Balantiocheilos ambusticauda* with a mean intensity of 1.53 per fish infected (Table 48).

Table 45 Intensity of *O. viverrini* metacercariae detected in freshwater fishes from Nikhom Kham Soi, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected OV	total metacercariae detected	Range (Min-Max)	Intensity
1	*Balantiocheilos ambusticauda*	122	9(7.37)	15	1-3	1.66

Ov, *O. viverrini*.

Table 46 Intensity of *H. taichui* metacercariae detected in freshwater fishes from Nikhom Kham Soi, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HT	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus gonionotus*	141	58(41.13)	78	1-2	1.34

Ht, *H. taichui*.

Table 47 Intensity of *H. pumilio* metacercariae detected in freshwater fishes from Nikhom Kham Soi, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Balantiocheilos ambusticauda*	122	8(6.55)	11	1-2	1.37

Hp, *Haplorchis pumilio*.

Table 48 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Nikom KomeSoi, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus gonionotus*	141	103(73.04)	289	1-9	2.8
2	*Balantiocheilos ambusticauda*	122	82(67.21)	107	1-5	1.3
3	Total	263	185 (70.34)	405	1-9	1.53

Hap, *Haplorchoides* sp.

4.6 Occurrence of fish borne trematode infections in freshwater fishes from Sakon Nakhon, Northeast of Thailand

Figure 35 Geographic mapping of freshwater cyprinoid fishes collected area from Sakon Nakhon, Northeast of Thailand (17°15 ′N 104°13′E) by ArcGIS 10.5.

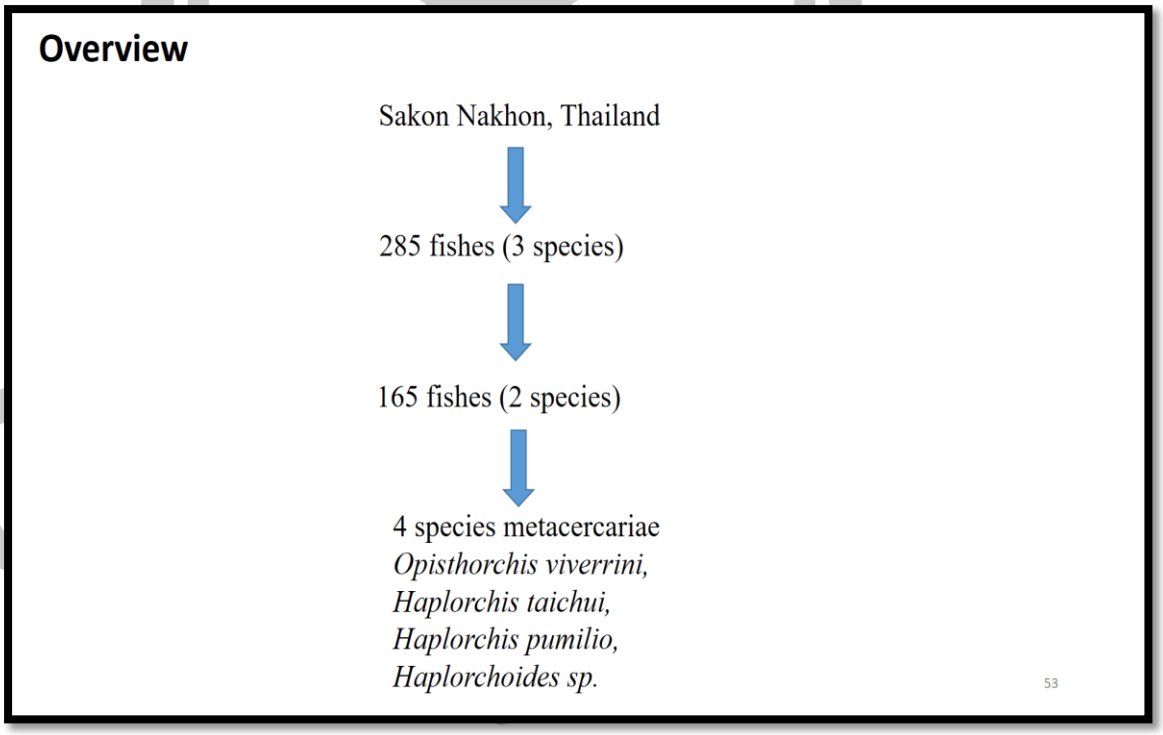


Figure 36 Flow chart of the overview of the research study from Sakon Nakhon, Northeast of Thailand

Osteochilus vittatus *Mystacoleucus marginatus* *Henicorhychus siamemsis*

Figure 37 Photos of fresh water cyprinoid fishes collected from Sakon Nakhon, Northeast of Thailand.

4.6.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Sakon Nakhon, Northeast of Thailand

In the total of 285 fishes, 165 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 57.89%. Among the 3 different species, 2 species of cyprinoid fishes, i.e., *Mystacoleucus marginatus, Henicorhychus siamemsis* were contaminated with the infective stage of trematode parasites, metacercariae (Table 2). The metacercariae of *O. viverrini* were found in *Mystacoleucus marginatus* (2.6 %, *n* = 153). The metacercariae of *H. taichui* were found infecting *Mystacoleucus marginatus, Henicorhychus siamemsis* species of fishes among the total 3 species of fishes. The prevalence of infection was found in *Mystacoleucus marginatus* (46.09 %, *n* = 153) and *Henicorhychus siamemsis* (35.38 %, *n* = 65). The metacercariae of *H. pumilio* were infected in *Henicorhychus siamemsis* (16.9 %, *n* = 65). The metacercariae *Haplorchoides* sp. were present in in *Mystacoleucus marginatus* (77.77%, *n* = 153), and *Henicorhychus siamemsis* (70.76 %, *n* = 65), respectively.

4.6.2 Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Sakon Nakhon, Northeast of Thailand

Simultaneous infections of one fish with two or more trematode species were recorded in some fish species, i.e., *Mystacoleucus marginatus, Henicorhychus siamemsis* (Table 49).

Three species of fish-borne trematode metacercariae, i.e., *O. viverrini*, *H. taichui*, *Haplorchoides* sp. simultaneously infected *Mystacoleucus marginatus* (Table 49). The fish species *Henicorhychus siamemsis*was infected with *H. taichui*, *H. pumilio*, *Haplorchoides* sp. (Table 49).

Table 49 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Sakon Nakhon, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Osteochilus vittatus*	67	0	_	_	_	_
2	*Mystacoleucus marginatus*	153	119 (77.77)	4 (2.6)	69 (45.09)	_	119 (77.77)
3	*Henicorhychus siamemsis*	65	46(70.76)	_	23(35.38)	11(16.9)	46(70.76)
	Total	285	165(57.89)				

4.6.3 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Sakon Nakhon, Northeast of Thailand

A total of 5 metacercariae of *O. viverrini* were detected from *Mystacoleucus marginatus* with a mean intensity of 1.25 per fish infected (Table 50). The metacercariae of *H. taichui* were collected in 92 (42.20 %) out of 218 fishes (2 species), i.e., *Mystacoleucus marginatus*, *Henicorhychus siamemsis* with a mean intensity of 1.81 per fish infected (Table 51). The metacercariae of *H. pumilio* were detected in 11 (16.9%) out of 65 in *Henicorhychus siamemsis* spieces of fishes, with a mean intensity of 1.8 per fish infected (Table 52). The metacercariae of *Haplorchoides* sp. were detected in 165 (75.68%) out of 218 fishes (2 species), i.e.,

Mystacoleucus marginatus, Henicorhychus siamemsis with a mean intensity of 2.59 per fish infected (Table 53).

Table 50 Intensity of *O. viverrini* metacercariae detected in freshwater fishes from Mae Ngat reservoir, Chiang Mai, Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected OV	total metacercariae detected	Range (Min-Max)	Intensity
1	*Mystacoleucus marginatus*	153	4 (2.6)	5	1-2	1.25

Ov, *O. viverrini*.

Table 51 Intensity of *H. taichui* metacercariae detected in freshwater fishes from Sakon Nakhon, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HT	total metacercariae detected	Range (Min-Max)	Intensity
1	*Mystacoleucus marginatus*	153	69 (45.09)	125	1-15	1.81
2	*Henicorhychus siamemsis*	65	23(35.38)	42	1-5	1.82
3	Total	218	92(42.20)	167	1-15	1.81

Ht, *H. taichui*.

Table 52 Intensity of *H. pumilio* metacercariae detected in freshwater fishes from Sakon Nakhon, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected	total metacercariae detected	Range (Min-Max)	Intensity
1	*Henicorhychus siamemsis*	65	11(16.9)	20	1-2	1.8

Hp, *Haplorchis pumilio*.

Table 53 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Sakon Nakhon, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Mystacoleucus marginatus*	153	119 (77.77)	496	1-12	4.16
2	*Henicorhychus siamemsis*	65	46(70.76)	69	1-7	1.5
	Total	218	165(75.68)	565	1-12	2.59

Hap, *Haplorchoides* sp.

4.7 Occurrence of fish borne trematode infections in freshwater fishes from Sisaket, Northeast of Thailand

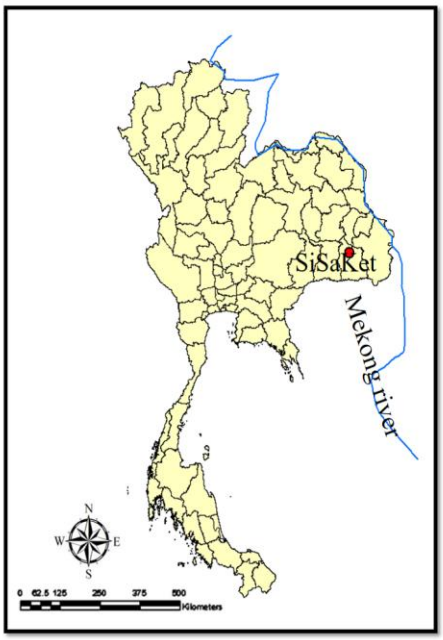


Figure 38 Geographic mapping of freshwater cyprinoid fishes collected area from Sisaket, Northeast of Thailand (15°12′N 104°32′E) by ArcGIS 10.5.

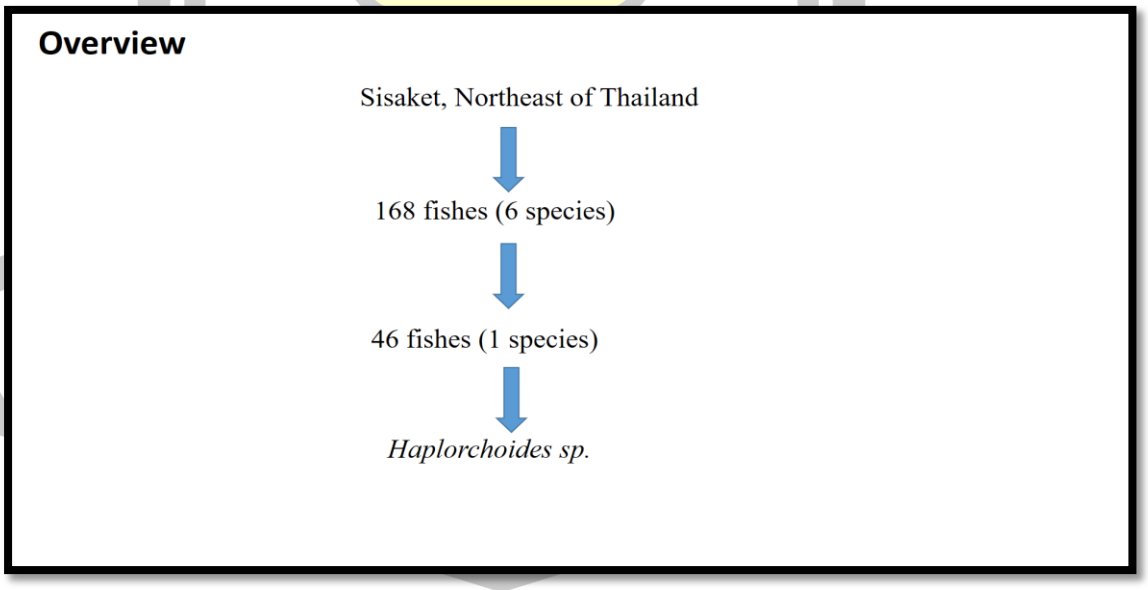


Figure 39 Flow chart of the overview of the research study from Sisaket, Northeast of Thailand

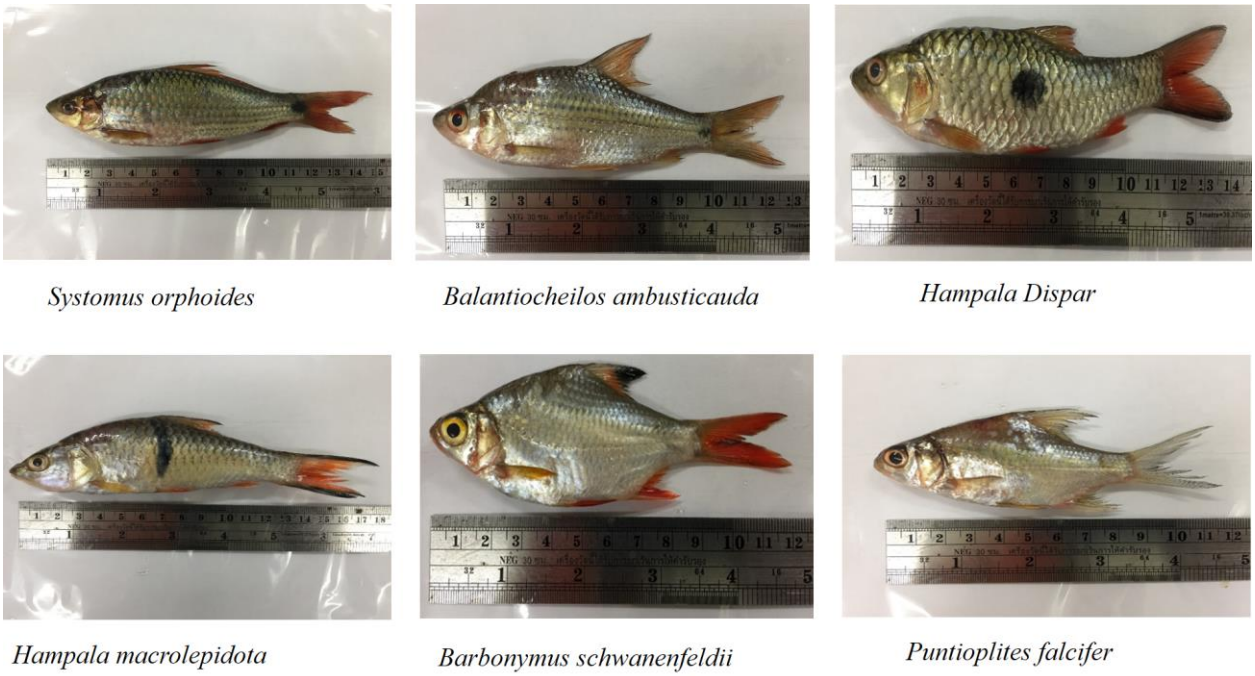


Figure 40 Photos of fresh water cyprinoid fishes collected from Sisaket, Northeast of Thailand

Table 54 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Sisaket, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Systomus orphoides*	24	0(0)	_	_	_	_
2	*Balantiocheilos ambusticauda*	30	0(0)	_	_	_	_
3	*Hampala Dispar*	10	0(0)	_	_	_	_
4	*Hampala macrolepidota*	10	0(0)	_	_	_	_

Table 54 (Continued)

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
5	*Barbonymus schwanenfeldii*	84	46(54.76)	_	_	_	46(54.76)
6	*Puntioplites falcifer*	10	0(0)	_	_	_	_
	Total	168	46(27.38)				

4.7.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Sisaket, Northeast of Thailand

In the total of 168 fishes, 46 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 27.38 %. Among the 6 different species, only *Barbonymus schwanenfeldii* was contaminated with the infective stage of trematode parasites, metacercariae (Table 54). The metacercariae *Haplorchoides* sp. was detected in Barbonymus *schwanenfeldii* (54.76%, *n* = 84).

4.7.2 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Sisaket, Northeast of Thailand

The metacercariae of *Haplorchoides* sp. were detected in *Barbonymus schwanenfeldii* species of fishes 46 (54.76%) out of 84 fishes with a mean intensity of 5.91 per fish infected (Table 55).

Table 55 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Sisaket, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Barbonymus schwanenfeldii*	84	46(54.76)	153	1-5	5.91

Hap, *Haplorchoides* sp.

4.8 Occurrence of fish borne trematode infections in freshwater fishes from Kalasin, Northeast of Thailand

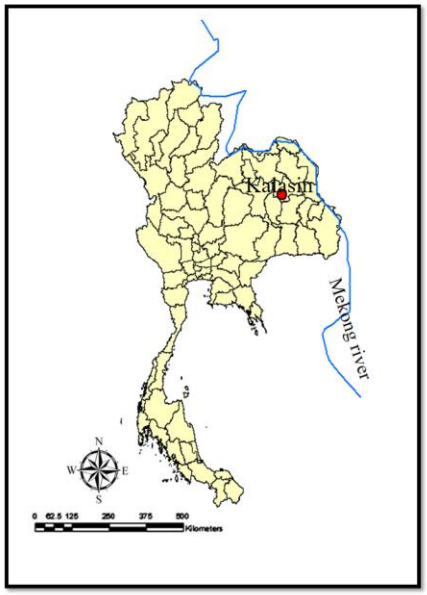


Figure 41 Geographic mapping of freshwater cyprinoid fishes collected area from Kalasin, Northeast of Thailand (16°44′N 103°51′E) by ArcGIS 10.5.

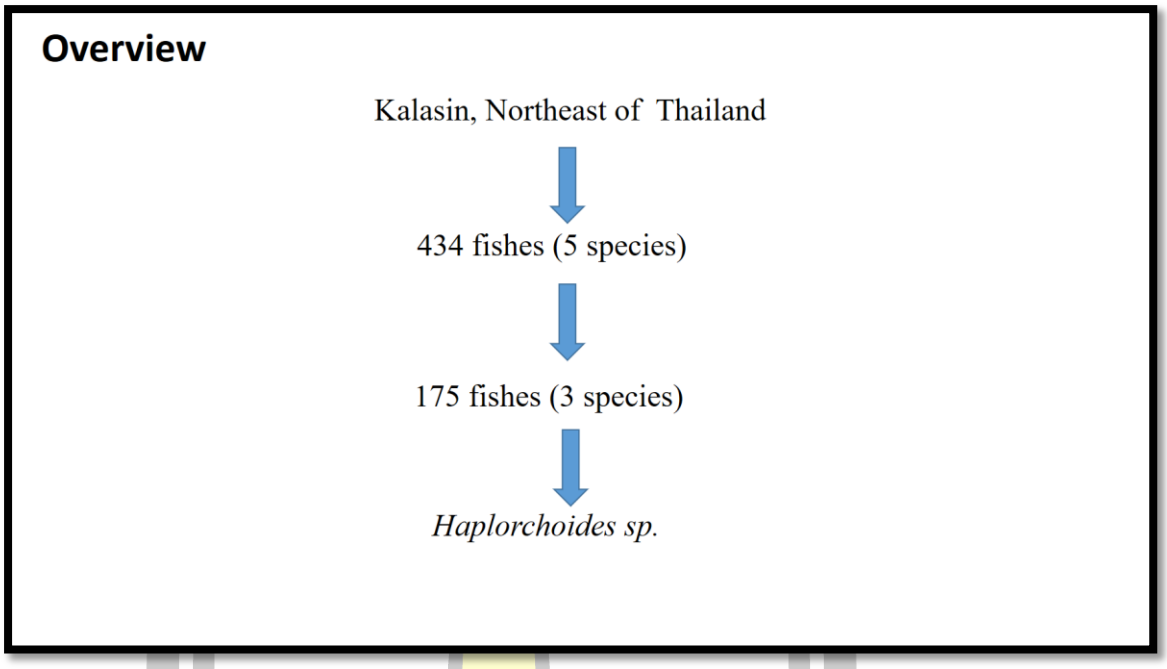


Figure 42 Flow chart of the overview of the research study from Kalasin, Northeast of Thailand

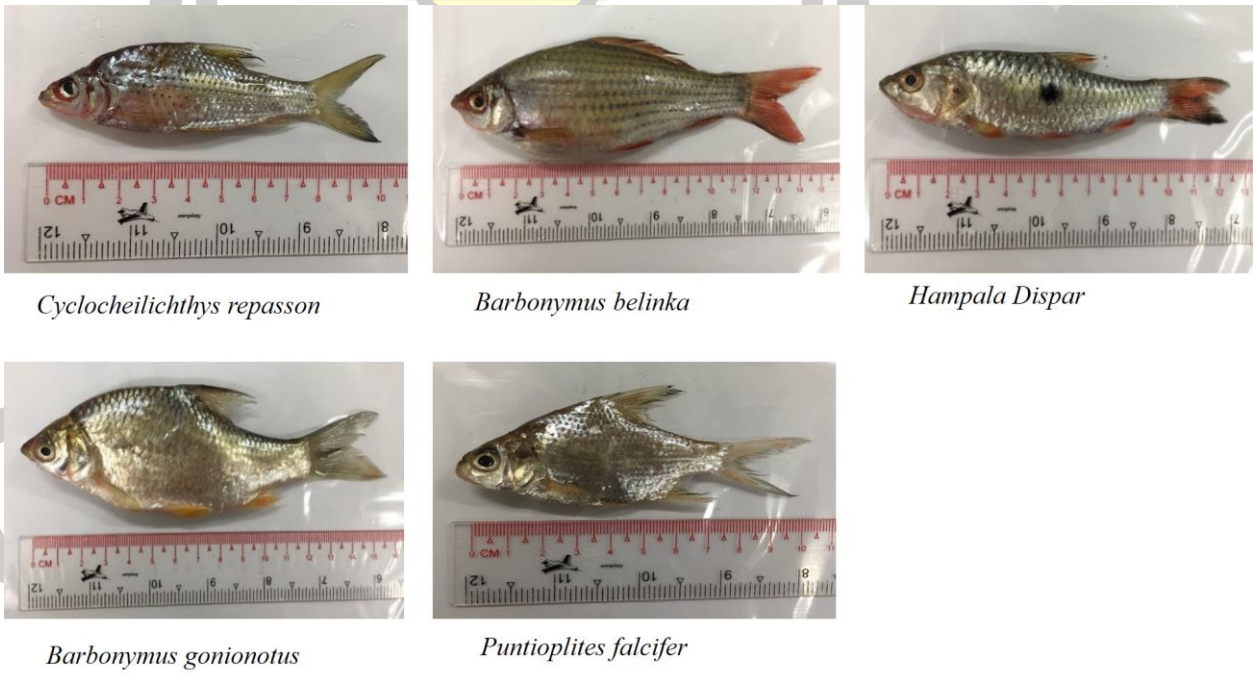


Figure 43 Photos of fresh water cyprinoid fishes collected from Kalasin, Northeast of Thailand

4.8.1 Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater fishes from Kalasin, Northeast of Thailand

In the total of 434 fishes, 175 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 40.32%. Among the 5 different species, 3 species of cyprinoid fishes, i.e. *Cyclocheilichthys repasson*, *Barbonymus gonionotus*, *Puntioplites falcifer* were contaminated with the infective stage of trematode parasites, metacercariae (Table 56). The metacercariae *Haplorchoides* sp. were present in *Cyclocheilichthys repasson* (76.61%, *n* = 124), *Barbonymus gonionotus* (35 %, n = 100), *Puntioplites falcifer C. repasson* (45 %, *n* = 100) respectively.

Table 56 Infection status and prevalence of fish-borne trematode infections in freshwater fish from Kalasin, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%)of fish infected with FBT	No. (%)of fish infected with OV	No. (%)of fish infected with HT	No. (%)of fish infected with HP	No. (%)of fish infected with HAP
1	*Cyclocheilichthys repasson*	124	95(76.61)	_	_	_	95(76.61)
2	*Barbonymus belinka*	60	0(0)	_	_	_	_
3	*Hampala Dispar*	50	0(0)	_	_	_	_
4	*Barbonymus gonionotus*	100	35(35)	_	_	_	35(35)
5	*Puntioplites falcifer*	100	45(45)	_	_	_	45(45)
	Total	434	175(40.32)				

4.8.2 Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Kalasin, Northeast of Thailand

The metacercariae of *Haplorchoides* sp. were detected in 175 (40.32 %) out of 434 fishes (3 species), i.e., *Cyclocheilichthys repasson*, *Barbonymus gonionotus*, *Puntioplites falcifer*, with a mean intensity of 1.64 per fish infected (Table 57).

Table 57 Intensity of *Haplorchoides sp.* metacercariae detected in freshwater fishes from Kalasin, Northeast of Thailand

No	Fish Species	No. of fish examined	No. (%) of fish infected HAP	total metacercariae detected	Range (Min-Max)	Intensity
1	*Cyclocheilichthys repasson*	124	95(76.61)	153	1-5	1.61
2	*Barbonymus gonionotus*	100	35(35)	73	1-3	2.08
3	*Puntioplites falcifer*	100	45(45)	61	1-4	1.3
4	Total	434	175(40.32)	287	1-5	1.64

Hap, *Haplorchoides* sp.

4.9 Morphology of detected metacercariae

Metacercariae of *O. viverrini* were elliptical, had nearly equal sized oral sucker and ventral sucker, brownish pigment granules scattered within the body and an O-shaped excretory bladder occupying the greater part of the posterior body (Figure 44).

H. taichui metacercariae were elliptical and had a baseball glove-shaped ventrogenital sac with rodlets and an O-shaped excretory bladder occupying large portion of posterior body (Figure 45).

H. pumilio metacercariae were elliptical and had deer horn-like minute spines arranged in 1–2 rows around the ventrogenital complex and an O-shaped excretory bladder occupying large portion of posterior body (Figure 46).

Haplorchoides sp. are nearly spherical, with a double layered cystic wall and have lance-shaped bodies, with a scale like spine on the body surface. Acetabulum with spines present and excretory bladder is saccular (Figure 47)

Figure 44 Photo of *Opisthorchis viverrini* metacercaria (scale bar- 30µm)

Figure 45 Photo of *Haplorchis taichui* metacercaria (scale bar- 30µm)

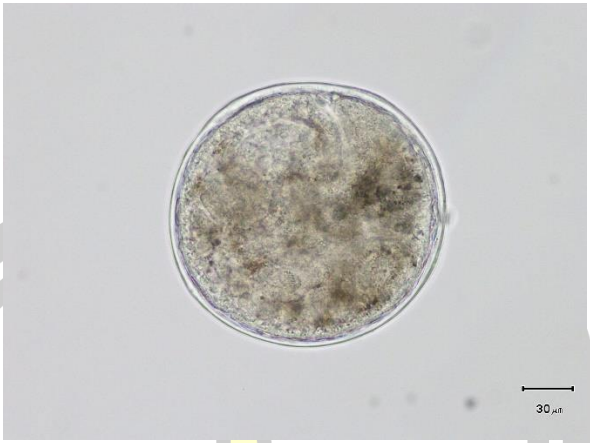


Figure 46 Photo of *Haplorchis pumilio* metacercaria (scale bar- 30µm)

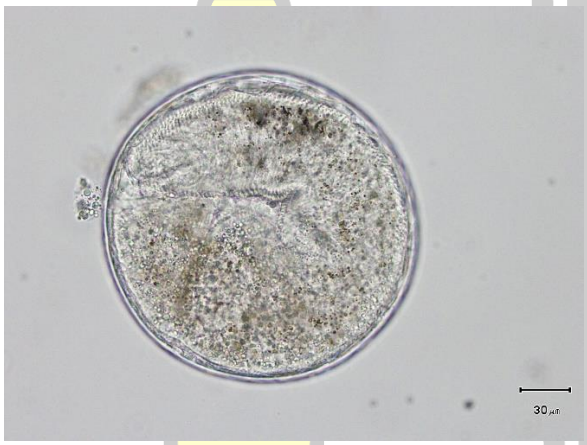


Figure 47 Photo of, *Haplorchoides* sp. metacercaria (scale bar- 30µm)

4.10 Geographic Information System of Fish- borne trematodes Metacercariae

The geographic information (latitude and longitude) associated with the infection rates among susceptible species of fresh water fish was recorded and built a geo-dataset for GIS development. Mapping of study locations from Thailand and Myanmar is created by using ArcGIS 10.5. (Figure 49). Geographic Information System showing Prevalence of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar with the population background by ArcGIS 10.5 (Figure 50). The size and scale of the red circles corresponds to the cumulative prevalence of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar and the back ground is showing population density (Figure

50). Geographic Information System showing Prevalence of detected metacercariae *ie; Opisthorchis viverrini, Haploris taichui, Haplorchis pumilio* and *Haplorchoides* sp. with the population density back ground (Figure 51). Figure – 4 is demonstrated that Geographic Information System showing *O.viverrini* in human from Myanmar and Northeast of Thailand collected data from reported articles with the associated population density created by ArcGIS 10.5.

A GIS database for the study of fish-borne metacercariae implemented using an ArcGIS Desktop program from the ESRI Company, Bangkok, Thailand. A geographic information system (GIS) is a framework for gathering and analyzing data. GIS integrates many types of data. It analyzes spatial location and organizes layers of information into visualizations using maps and 3D scenes. With this unique capability, GIS reveals deeper insights into data, by helping users make smarter decisions. In our study, we investigated environmental factors that influence to fish-borne metacercariae, the GIS database was overlaid with population density and precipitation GIS datasets. Geographic coordinates of each area are determined with a Global Positioning System.

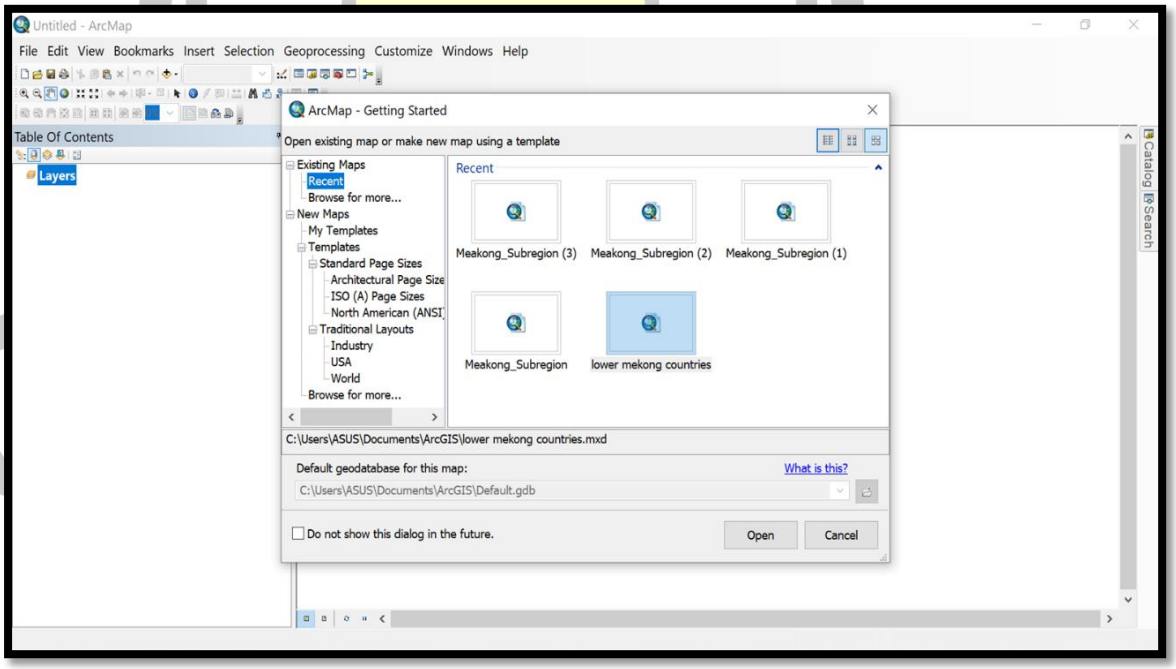
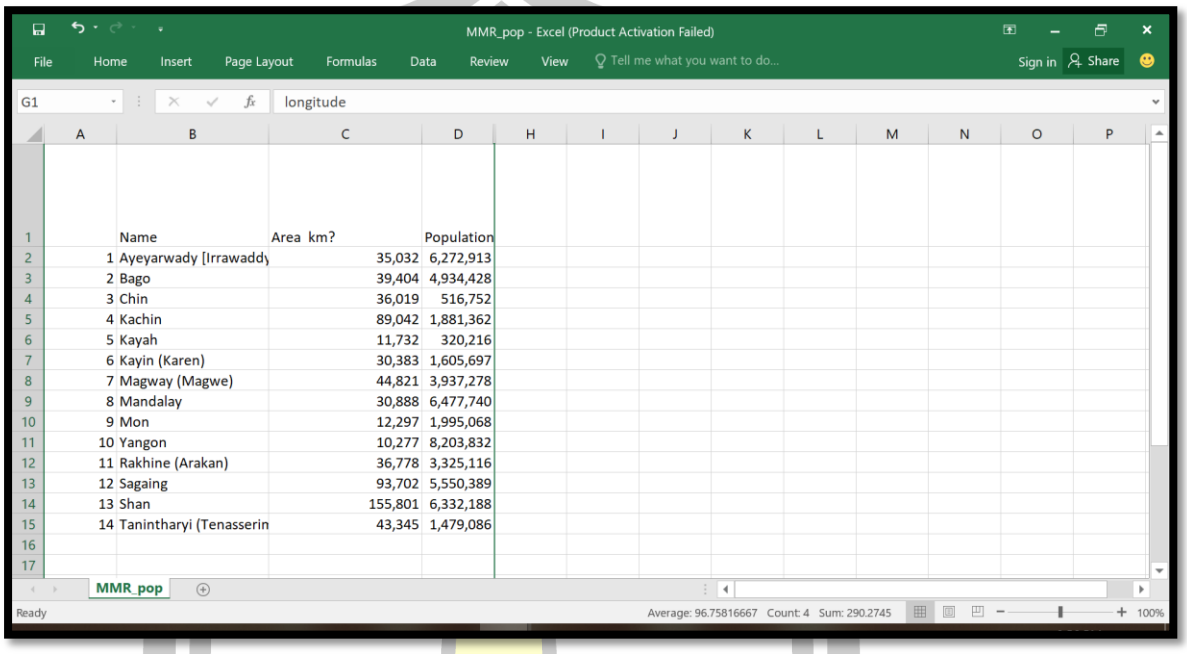


Figure 48 showing the GIS database using an ArcGIS Desktop program from the ESRI Company, Bangkok, Thailand

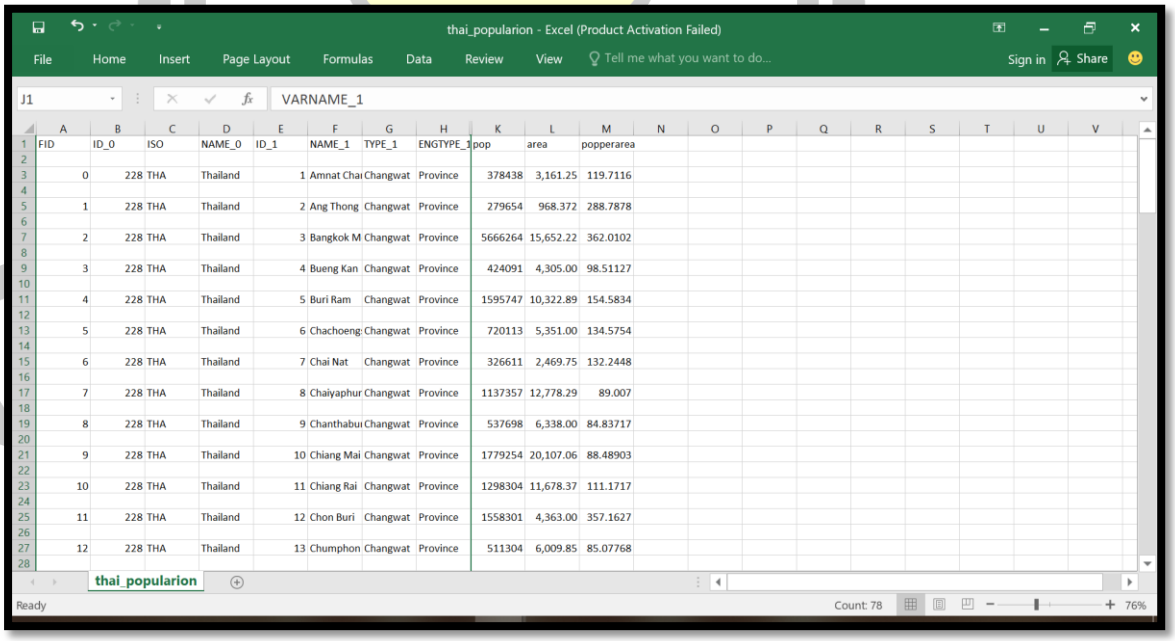
Table 58 Showing the population density in Myanmar
(https://myanmar.unfpa.org/sites/default/files/pub-pdf/MyanmarCensusAtlas_lowres.pdf)



MMR_pop - Excel (Product Activation Failed)

	Name	Area km?	Population
1	Ayeyarwady [Irrawaddy	35,032	6,272,913
2	Bago	39,404	4,934,428
3	Chin	36,019	516,752
4	Kachin	89,042	1,881,362
5	Kayah	11,732	320,216
6	Kayin (Karen)	30,383	1,605,697
7	Magway (Magwe)	44,821	3,937,278
8	Mandalay	30,888	6,477,740
9	Mon	12,297	1,995,068
10	Yangon	10,277	8,203,832
11	Rakhine (Arakan)	36,778	3,325,116
12	Sagaing	93,702	5,550,389
13	Shan	155,801	6,332,188
14	Tanintharyi (Tenasserin	43,345	1,479,086

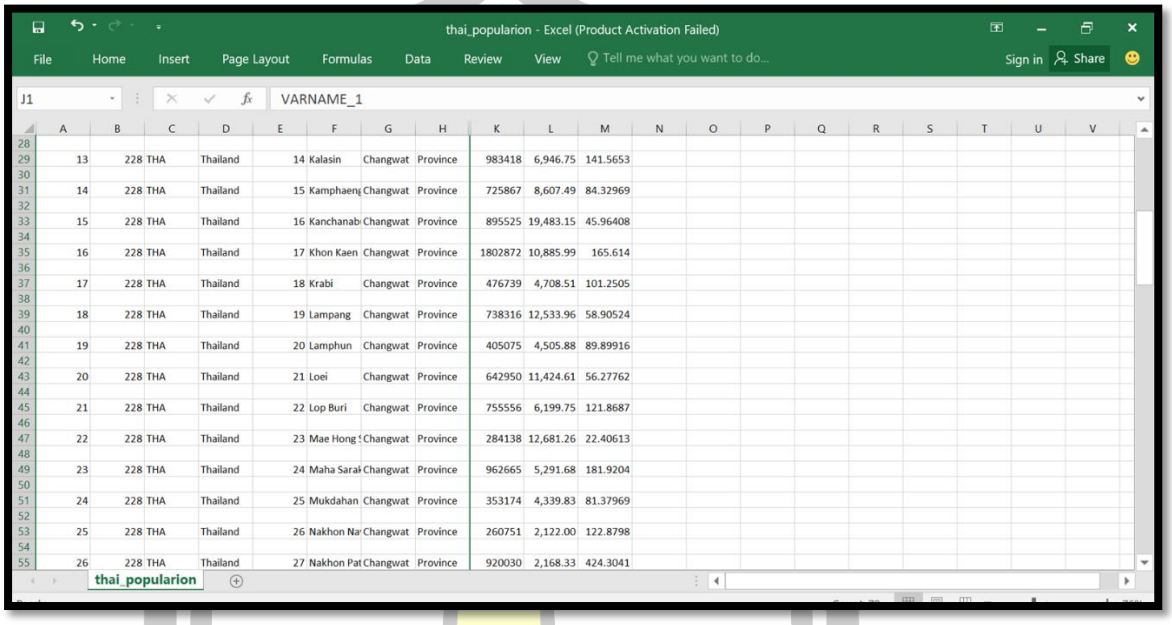
Table 59 Showing the population density in Thailand (a)
[https://stat.dopa.go.th/stat/statnew/statMenu/newStat/home.php]



thai_populario - Excel (Product Activation Failed)

FID	ID_0	ISO	NAME_0	ID_1	NAME_1	TYPE_1	ENGTYPE_1	pop	area	popperarea
0	228	THA	Thailand	1	Amnat Chai	Changwat	Province	378438	3,161.25	119.7116
1	228	THA	Thailand	2	Ang Thong	Changwat	Province	279654	968.372	288.7878
2	228	THA	Thailand	3	Bangkok M	Changwat	Province	5666264	15,652.22	362.0102
3	228	THA	Thailand	4	Bueng Kan	Changwat	Province	424091	4,305.00	98.51127
4	228	THA	Thailand	5	Buri Ram	Changwat	Province	1595747	10,322.89	154.5834
5	228	THA	Thailand	6	Chachoeng	Changwat	Province	720113	5,351.00	134.5754
6	228	THA	Thailand	7	Chai Nat	Changwat	Province	326611	2,469.75	132.2448
7	228	THA	Thailand	8	Chaiyaphur	Changwat	Province	1137357	12,778.29	89.007
8	228	THA	Thailand	9	Chanthabu	Changwat	Province	537698	6,338.00	84.83717
9	228	THA	Thailand	10	Chiang Mai	Changwat	Province	1779254	20,107.06	88.48903
10	228	THA	Thailand	11	Chiang Rai	Changwat	Province	1298304	11,678.37	111.1717
11	228	THA	Thailand	12	Chon Buri	Changwat	Province	1558301	4,363.00	357.1627
12	228	THA	Thailand	13	Chumphon	Changwat	Province	511304	6,009.85	85.07768

Table 60 Showing the population density in Thailand (b)
[https://stat.dopa.go.th/stat/statnew/statMenu/newStat/home.php]



thai_popularion - Excel (Product Activation Failed)

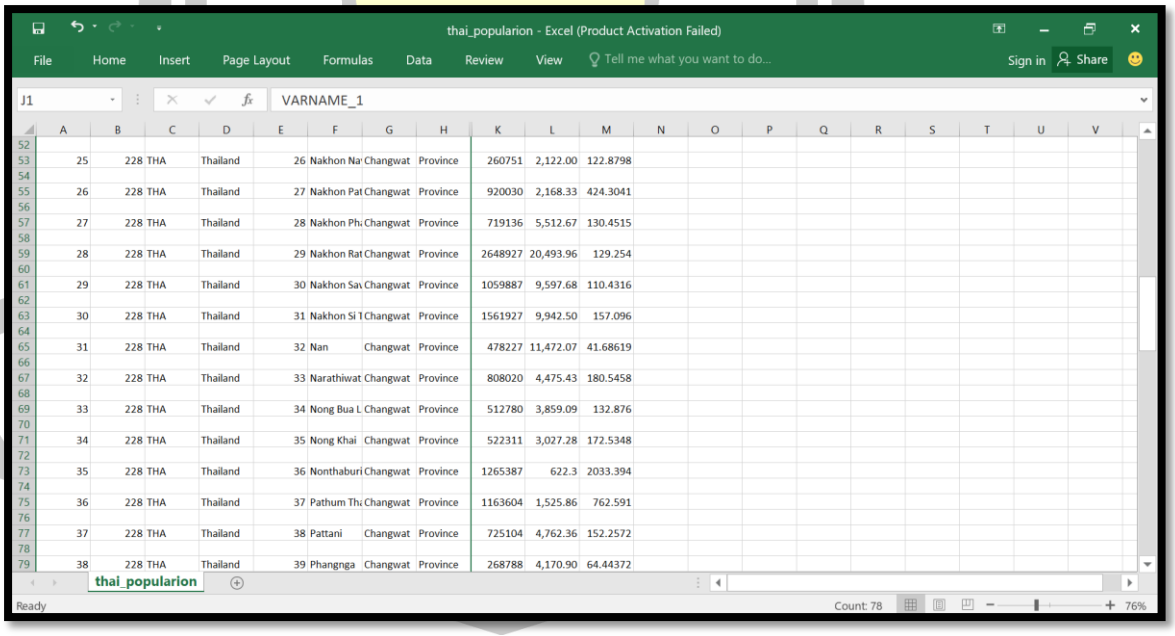
File Home Insert Page Layout Formulas Data Review View Tell me what you want to do... Sign in Share

J1 VARNAME_1

	A	B	C	D	E	F	G	H	K	L	M
28											
29	13	228	THA	Thailand	14	Kalasin	Changwat	Province	983418	6,946.75	141.5653
30											
31	14	228	THA	Thailand	15	Kamphaeng	Changwat	Province	725867	8,607.49	84.32969
32											
33	15	228	THA	Thailand	16	Kanchanab	Changwat	Province	895525	19,483.15	45.96408
34											
35	16	228	THA	Thailand	17	Khon Kaen	Changwat	Province	1802872	10,885.99	165.614
36											
37	17	228	THA	Thailand	18	Krabi	Changwat	Province	476739	4,708.51	101.2505
38											
39	18	228	THA	Thailand	19	Lampang	Changwat	Province	738316	12,533.96	58.90524
40											
41	19	228	THA	Thailand	20	Lamphun	Changwat	Province	405075	4,505.88	89.89916
42											
43	20	228	THA	Thailand	21	Loei	Changwat	Province	642950	11,424.61	56.27762
44											
45	21	228	THA	Thailand	22	Lop Buri	Changwat	Province	755556	6,199.75	121.8687
46											
47	22	228	THA	Thailand	23	Mae Hong S	Changwat	Province	284138	12,681.26	22.40613
48											
49	23	228	THA	Thailand	24	Maha Sarak	Changwat	Province	962665	5,291.68	181.9204
50											
51	24	228	THA	Thailand	25	Mukdahan	Changwat	Province	353174	4,339.83	81.37969
52											
53	25	228	THA	Thailand	26	Nakhon Na	Changwat	Province	260751	2,122.00	122.8798
54											
55	26	228	THA	Thailand	27	Nakhon Pat	Changwat	Province	920030	2,168.33	424.3041

thai_popularion

Table 61 Showing the population density in Thailand (c)
[https://stat.dopa.go.th/stat/statnew/statMenu/newStat/home.php]



thai_popularion - Excel (Product Activation Failed)

File Home Insert Page Layout Formulas Data Review View Tell me what you want to do... Sign in Share

J1 VARNAME_1

	A	B	C	D	E	F	G	H	K	L	M
52											
53	25	228	THA	Thailand	26	Nakhon Na	Changwat	Province	260751	2,122.00	122.8798
54											
55	26	228	THA	Thailand	27	Nakhon Pat	Changwat	Province	920030	2,168.33	424.3041
56											
57	27	228	THA	Thailand	28	Nakhon Ph	Changwat	Province	719136	5,512.67	130.4515
58											
59	28	228	THA	Thailand	29	Nakhon Rat	Changwat	Province	2648927	20,493.96	129.254
60											
61	29	228	THA	Thailand	30	Nakhon Sa	Changwat	Province	1059887	9,597.68	110.4316
62											
63	30	228	THA	Thailand	31	Nakhon Si T	Changwat	Province	1561927	9,942.50	157.096
64											
65	31	228	THA	Thailand	32	Nan	Changwat	Province	478227	11,472.07	41.68619
66											
67	32	228	THA	Thailand	33	Narathiwat	Changwat	Province	808020	4,475.43	180.5458
68											
69	33	228	THA	Thailand	34	Nong Bua L	Changwat	Province	512780	3,859.09	132.876
70											
71	34	228	THA	Thailand	35	Nong Khai	Changwat	Province	522311	3,027.28	172.5348
72											
73	35	228	THA	Thailand	36	Nonthaburi	Changwat	Province	1265387	622.3	2033.394
74											
75	36	228	THA	Thailand	37	Pathum Th	Changwat	Province	1163604	1,525.86	762.591
76											
77	37	228	THA	Thailand	38	Pattani	Changwat	Province	725104	4,762.36	152.2572
78											
79	38	228	THA	Thailand	39	Phangnga	Changwat	Province	268788	4,170.90	64.44372

thai_popularion

Ready Count: 78 76%

Table 62 Showing the population density in Thailand (d)
[https://stat.dopa.go.th/stat/statnew/statMenu/newStat/home.php]

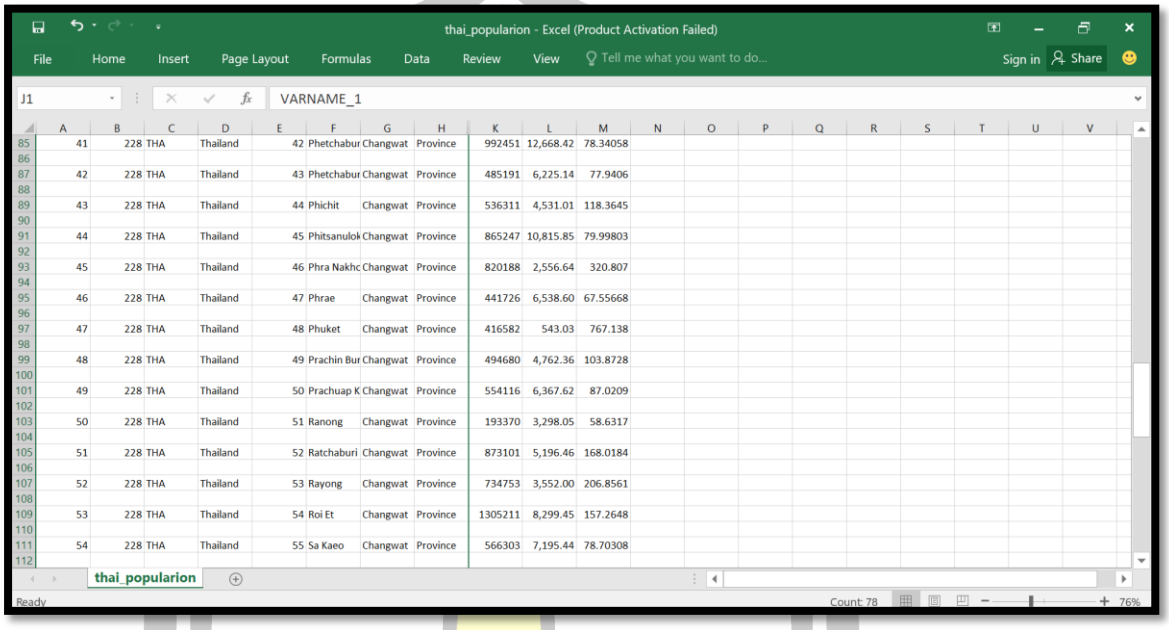


Table 63 Showing the population density in Thailand (e)
[https://stat.dopa.go.th/stat/statnew/statMenu/newStat/home.php]

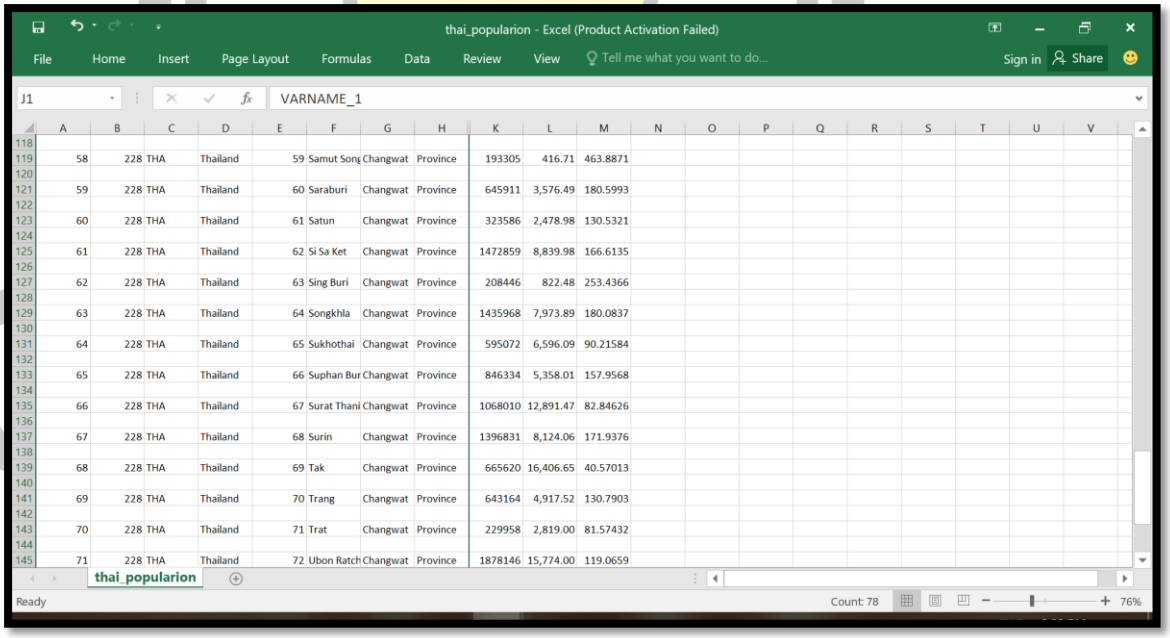
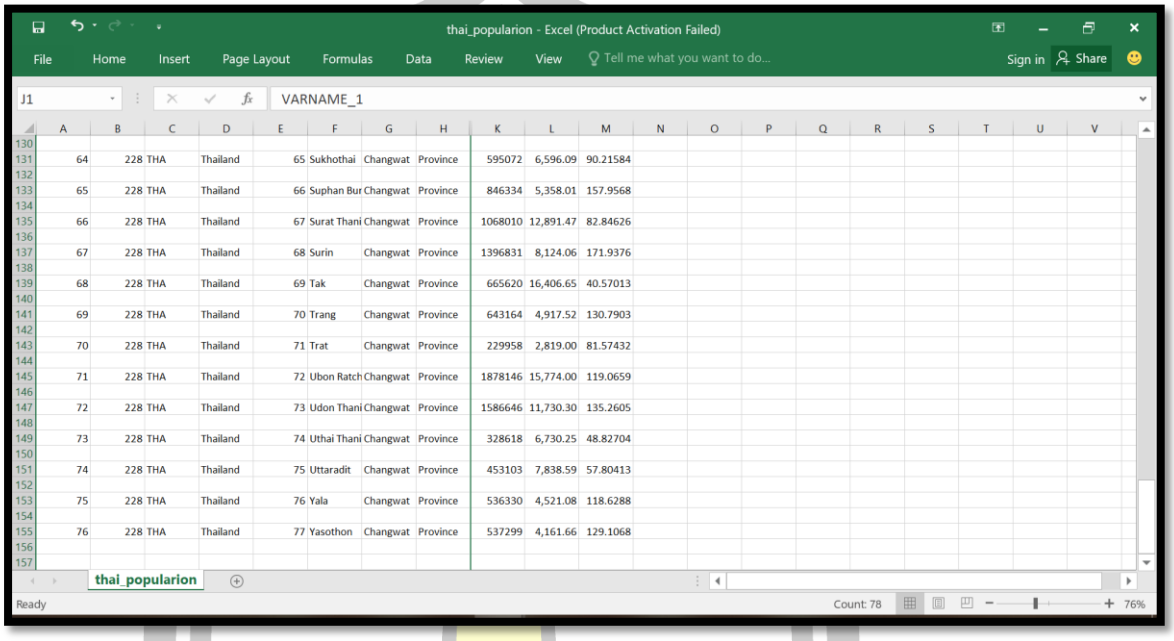


Table 64 Showing the population density in Thailand (f)
[https://stat.dopa.go.th/stat/statnew/statMenu/newStat/home.php]



thai_popularion - Excel (Product Activation Failed)

File Home Insert Page Layout Formulas Data Review View Tell me what you want to do... Sign in Share

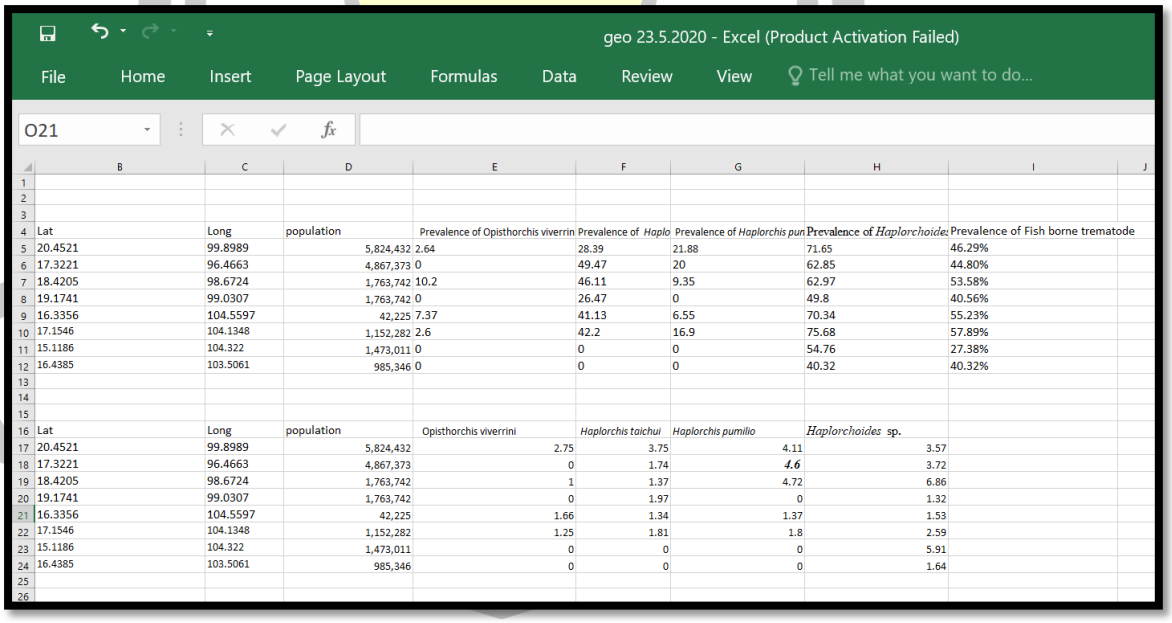
J1 VARNAME_1

	A	B	C	D	E	F	G	H	K	L	M	N	O	P	Q	R	S	T	U	V
130																				
131	64	228	THA	Thailand	65	Sukhothai	Changwat	Province	595072	6,596.09	90.21584									
132																				
133	65	228	THA	Thailand	66	Suphan Bur	Changwat	Province	846334	5,358.01	157.9568									
134																				
135	66	228	THA	Thailand	67	Surat Thani	Changwat	Province	1068010	12,891.47	82.84626									
136																				
137	67	228	THA	Thailand	68	Surin	Changwat	Province	1396831	8,124.06	171.9376									
138																				
139	68	228	THA	Thailand	69	Tak	Changwat	Province	665620	16,406.65	40.57013									
140																				
141	69	228	THA	Thailand	70	Trang	Changwat	Province	643164	4,917.52	130.7903									
142																				
143	70	228	THA	Thailand	71	Trat	Changwat	Province	229958	2,819.00	81.57432									
144																				
145	71	228	THA	Thailand	72	Ubon Ratch	Changwat	Province	1878146	15,774.00	119.0659									
146																				
147	72	228	THA	Thailand	73	Udon Thani	Changwat	Province	1586646	11,730.30	135.2605									
148																				
149	73	228	THA	Thailand	74	Uthai Thani	Changwat	Province	328618	6,730.25	48.82704									
150																				
151	74	228	THA	Thailand	75	Uttaradit	Changwat	Province	453103	7,838.59	57.80413									
152																				
153	75	228	THA	Thailand	76	Yala	Changwat	Province	536330	4,521.08	118.6288									
154																				
155	76	228	THA	Thailand	77	Yasothon	Changwat	Province	537299	4,161.66	129.1068									
156																				
157																				

thai_popularion

Ready Count: 78 76%

Table 65 showing Prevalence and intensity of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar



geo 23.5.2020 - Excel (Product Activation Failed)

File Home Insert Page Layout Formulas Data Review View Tell me what you want to do...

O21

	B	C	D	E	F	G	H	I	J
1									
2									
3									
4	Lat	Long	population	Prevalence of Opisthorchis viverrin	Prevalence of *Haplo*	Prevalence of *Haplorchis pun*	Prevalence of *Haplorchoides*	Prevalence of Fish borne trematode	
5	20.4521	99.8989	5,824,432	2.64	28.39	21.88	71.65	46.29%	
6	17.3221	96.4663	4,867,373	0	49.47	20	62.85	44.80%	
7	18.4205	98.6724	1,763,742	10.2	46.11	9.35	62.97	53.58%	
8	19.1741	99.0307	1,763,742	0	26.47	0	49.8	40.56%	
9	16.3356	104.5597	42,225	7.37	41.13	6.55	70.34	55.23%	
10	17.1546	104.1348	1,152,282	2.6	42.2	16.9	75.68	57.89%	
11	15.1186	104.322	1,473,011	0	0	0	54.76	27.38%	
12	16.4385	103.5061	985,346	0	0	0	40.32	40.32%	
13									
14									
15									
16	Lat	Long	population	Opisthorchis viverrini	*Haplorchis taichui*	*Haplorchis pumilio*	*Haplorchoides* sp.		
17	20.4521	99.8989	5,824,432	2.75	3.75	4.11	3.57		
18	17.3221	96.4663	4,867,373	0	1.74	***4.6***	3.72		
19	18.4205	98.6724	1,763,742	1	1.37	4.72	6.86		
20	19.1741	99.0307	1,763,742	0	1.97	0	1.32		
21	16.3356	104.5597	42,225	1.66	1.34	1.37	1.53		
22	17.1546	104.1348	1,152,282	1.25	1.81	1.8	2.59		
23	15.1186	104.322	1,473,011	0	0	0	5.91		
24	16.4385	103.5061	985,346	0	0	0	1.64		
25									
26									

Table 66 showing the Population Density and locations of 8 study areas

No	Locations	Population density	Latitude	Longitude	References
1	Tachileik, Shan state, Myanmar	5,824,432	20.4521	99.8989	https://en.wikipedia.org/wiki/Shan_State
2	Bago, Myanmar	4,867,373	17.3221	96.4663	https://en.wikipedia.org/wiki/Bago_Region
3	Chom Thong District, Chaing Mai, Thailand	1,763,742	18.4205	98.6724	https://en.wikipedia.org/wiki/Chiang_Mai_Province
4	Mae Ngat reservoir, Chaing Mai, Thailand	1,763,742	19.1741	99.0307	https://en.wikipedia.org/wiki/Chiang_Mai_Province
5	Nikhom Kham Soi, Thailand	42,225	16.3356	104.5597	https://en.wikipedia.org/wiki/Nikhom_Kham_Soi_District
6	Sakon Nakhon, Thailand	1,152,282	17.1546	104.1348	https://en.wikipedia.org/wiki/Sakon_Nakhon_Province
7	Sisaket, Thailand	1,473,011	15.1186	104.322	https://en.wikipedia.org/wiki/Sisaket_Province
8	Kalasin, Thailand	985,346	16.4385	103.5061	https://en.wikipedia.org/wiki/Kalasin_Province

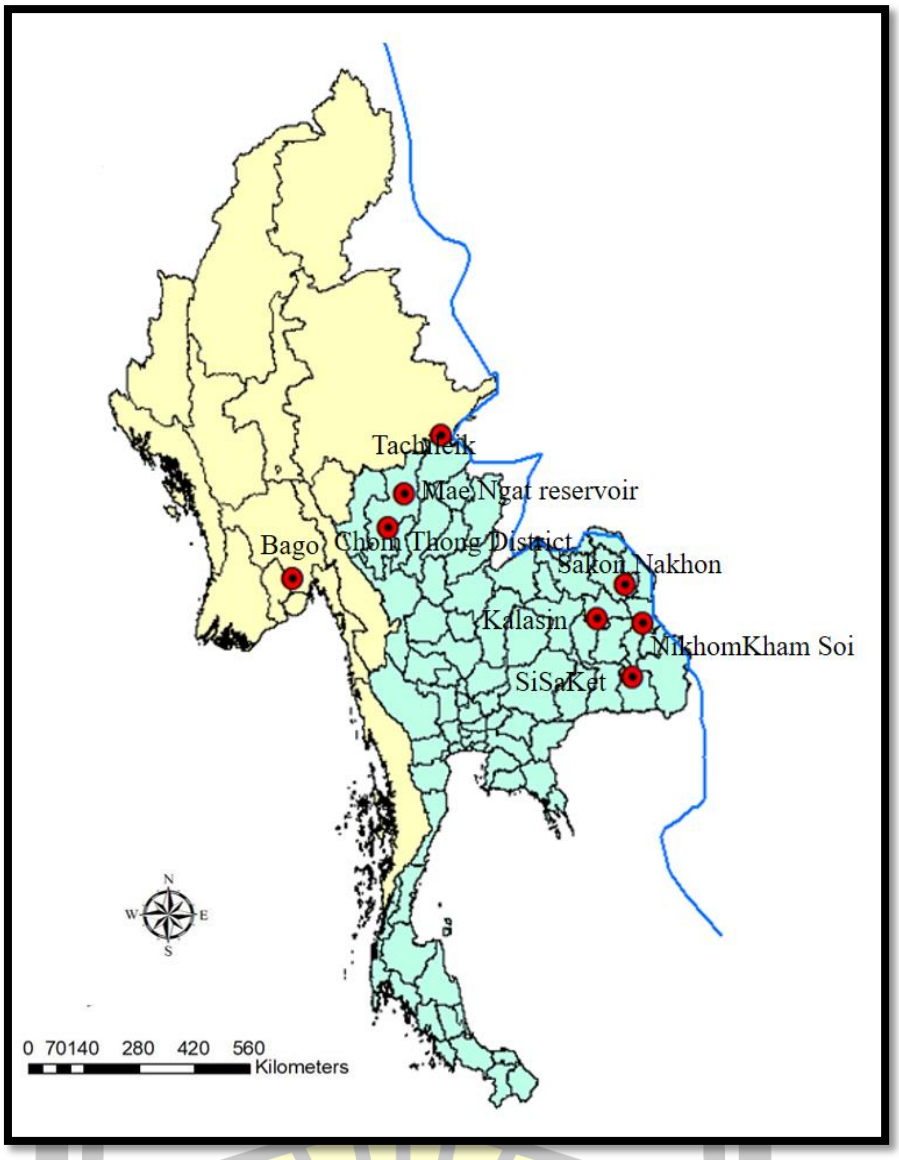


Figure 49 Map of Thailand and Myanmar showing the study areas created by using ArcGIS 10.5.

4.10.1 Geographic Information System showing Prevalence of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar

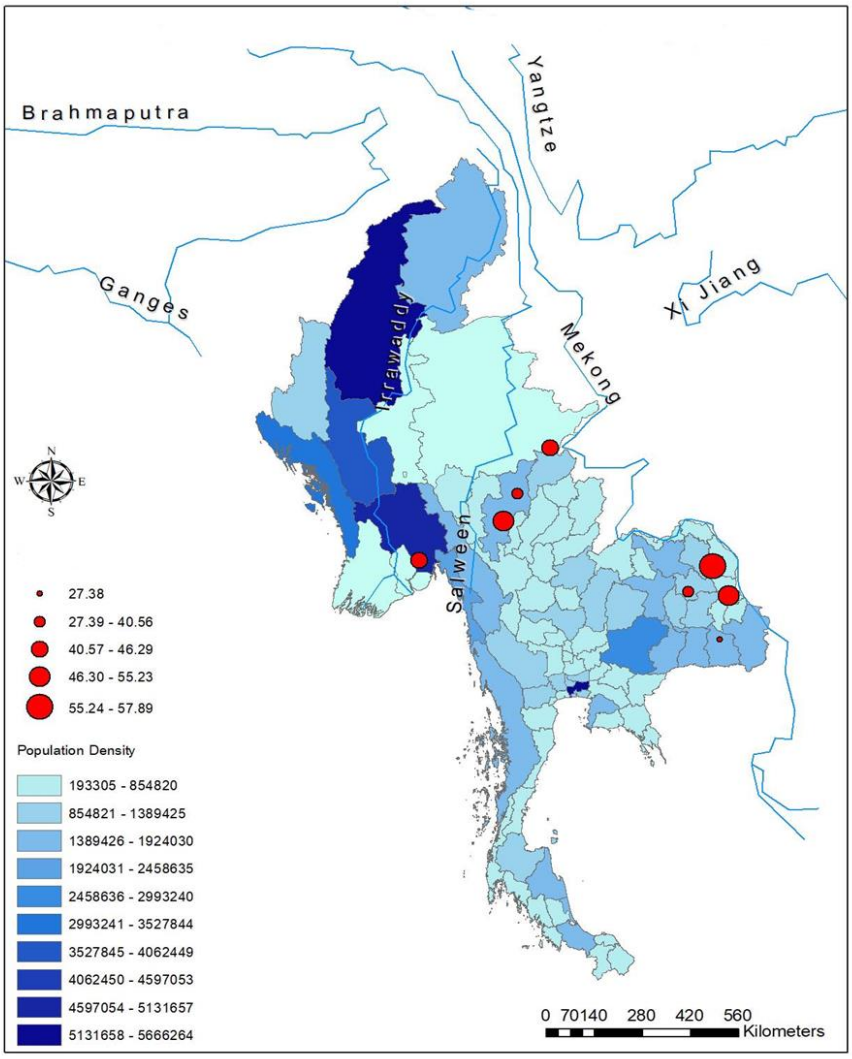


Figure 50 Geographic Information System showing Prevalence of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar with the population background by ArcGIS 10.5.

Table 67 Total Number of fishes and Prevalence of fish borne trematode infections collected from Myanmar and Thailand

No	Location	Total No of Fishes examined	Total Fish Species	Positive Fish Species	Positive number of Fishes	Negative number of Fishes	Prevalence of FZT
1	Tachileik	689	12	9	319	370	46.29%
2	Bago	125	4	3	56	69	44.80%
3	Chom Thong	530	8	4	284	246	53.58%
4	Mae Ngat	318	5	3	129	189	40.56%
5	Nikhom kham soi	335	5	2	185	150	55.23%
6	Sakon Nakhon	285	3	2	165	120	57.89%
7	Sisaket	168	6	1	46	122	27.38%
8	Kalasin	434	5	3	175	259	40.32%
	Total	2884	48	27	1359	1525	47.12%

4.10.2 Geographic Information System showing Prevalence of detected metacercariae from North and Northeast of Thailand and Myanmar.

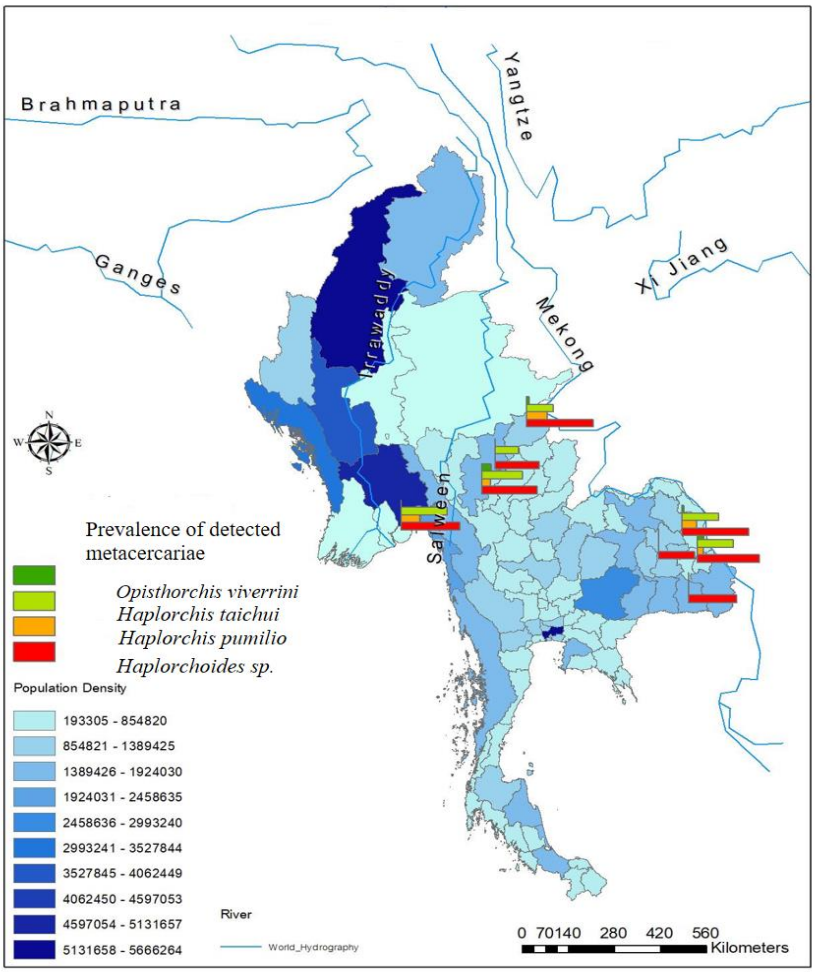


Figure 51 Geographic Information System showing Prevalence of detected metacercariae from North and Northeast of Thailand and Myanmar with the population background by ArcGIS 10.5.

4.10.3 Geographic Information System showing Prevalence of detected metacercariae *ie; Opisthorchis viverrini*, *Haploris taichui*, *Haplorchis pumilio* and *Haplorchoides* sp.

Table 68 Prevalence of detected metacercariae from North and Northeast of Thailand and Myanmar

Location	Prevalence of *Opisthorchis viverrini*	Prevalence of *Haplorchis taichui*	Prevalence of *Haplorchis pumilio*	Prevalence of *Haplorchoides sp.*
Tachileik, Mekong region of Myanmar	2.64	28.39	21.88	71.65
Bago, central region, Myanmar	0	49.47	20	62.85
Chom Thong District, Chaing Mai, North of Thailand	10.2	46.11	9.35	62.97
Mae Ngat reservoir, Chaing Mai, North of Thailand	0	26.47	0	49.8
Nikhom Kham Soi, Northeast of Thailand	7.37	41.13	6.55	70.34
Sakon Nakhon, Northeast of Thailand	2.6	42.2	16.9	75.68
Sisaket, Northeast of Thailand	0	0	0	54.76
Kalasin, Northeast of Thailand	0	0	0	40.32

4.10.4 Geographic Information System showing Intensity of detected metacercariae from North and Northeast of Thailand and Myanmar

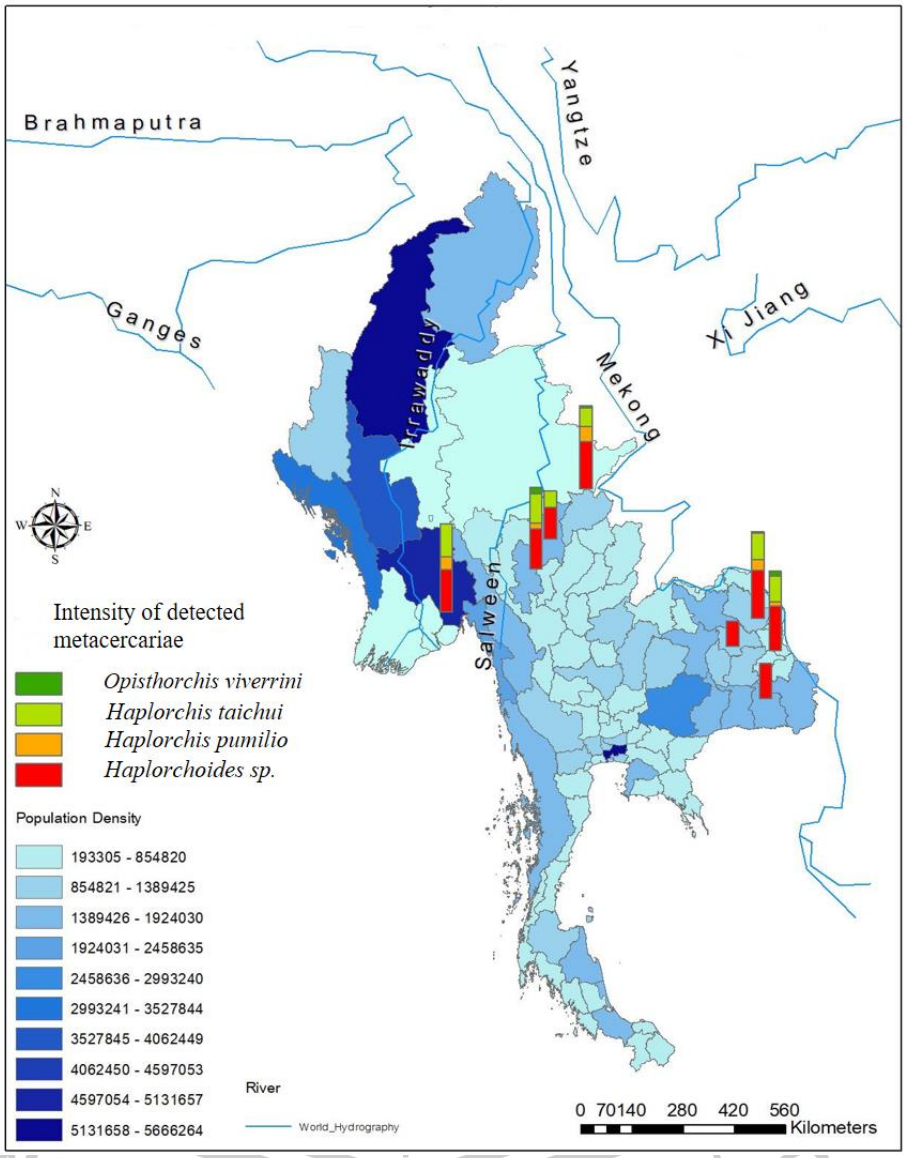


Figure 52 Geographic Information System showing Intensity of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar with the population background by ArcGIS 10.5.

Table 69 Intensity of fish-borne Trematode Metacercariae from North and Northeast of Thailand and Myanmar

Location	Intensity of *Opisthorchis viverrini*	Intensity of *Haplorchis taichui*	Intensity of *Haplorchis pumilio*	Intensity of *Haplorchoides sp.*
Tachileik, Mekong region of Myanmar	2.75	3.75	4.11	3.57
Bago, central region, Myanmar	0	2.74	4.6	3.72
Chom Thong District, Chaing Mai, North of Thailand	1	1.37	4.72	6.86
Mae Ngat reservoir, Chaing Mai, North of Thailand	0	1.97	0	1.32
Nikhom Kham Soi, Northeast of Thailand	1.66	1.34	1.37	1.53
Sakon Nakhon, Northeast of Thailand	1.25	1.81	1.80	2.59
Sisaket, Northeast of Thailand	0	0	0	5.91
Kalasin, Northeast of Thailand	0	0	0	1.64

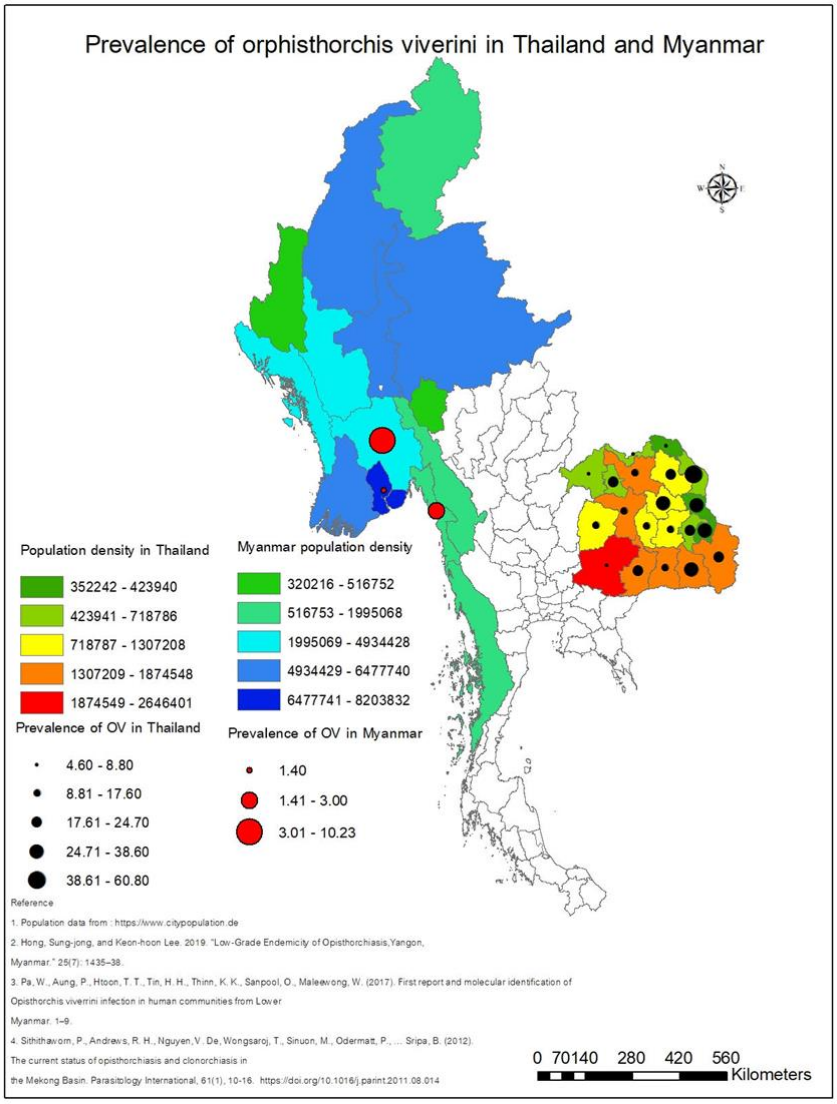


Figure 53 Geographic Information System showing *Opisthorchis viverrini* in human from Myanmar and Northeast of Thailand collected data from reported articles and created by ArcGIS 10.5.

CHAPTER 5

DISCUSSION

Freshwater cyprinid fishes are the second intermediate host of trematode infection such as small liver flukes and minute intestinal flukes. Zoonotic trematode infected fishes are found throughout many parts of lower Mekong basin countries Thailand (Nithikathkul, 2008) (Wongsawad et al., 2012) (Pinlaor et al., 2013), Laos (Sripa et al., 2011) (Sithithaworn et al., 2007) (Wongratanacheewin et al., 2003), Cambodia (Touch et al., 2013) (Boonmekam et al., 2016), Vietnam (Sohn WM., 2009) (Van et al., 2009), Central Region of Myanmar (Pa et al., 2017) (Chai et al., 2017) (Hong et al 2019). An important public health problem of the spread of fish borne trematodes (FBT) are being highly alarmed the countries of lower Mekong basin (Do et al., 2007) (Saenphet et al., 2008) (Lovis et al., 2009) (Sithithaworn et al., 2003) (Chai et al., 2017) Wattanayingcharoenchai et al., 2011; Jeon et al., 2012).Nevertheless, the contaminated fish species are different in each region.

A total of 2884 fishes (48 species) were investigated from total 8 locations from Mekong region of Myanmar, central region of Myanmar, north of Thailand and northeast of Thailand ie; Tachileik, Bago, Chom Thong, Mae Ngat, NikhomKham Soi, Sakon Nakhon, Sisaket, Kalasin.1359 fishes (27 species) were positive with fish borne trematode matacercariae infections. Four Species of fish borne trematode metacercariae i.e.; small liver fluke, *Opisthorchis viverrini*, minute intestinal flukes *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchoides sp.* were detected in this study. We have demonstrated for the first time the presence of *O. viverrini* metacercariae in cyprinoid fish, *Cyclocheilichthys repasson*, in the Tachileik, Myanmar. The prevalence is 2.64 % and the intensity is 2.75. Opisthorchiasis is best known in or near the basin of the lower Mekong River. Tachileik is a town in the Shan State situated in Lower Mekong basin of Myanmar which is also involved in the Golden Triangle that is the area where the borders of Thailand, Laos, and Myanmar meet at the confluence of the Ruak and Mekong rivers. There is no report for fish borne trematode matercerceriae infection lower Mekong region of Myanmar. We

provide information to fill the gap knowledge on trematode matercerceriae infection in second intermediate host in lower Mekong region of Myanmar.

From Tachileik, Mekong region of Myanmar, we collected 689 fishes (12 species) and found 319 fishes (8 species) and detected 4 species metacercariae; *Opisthorchis viverrini, Haplorchis taichui, Haplorchis pumilio, Haplorchoides sp*. The overall prevalence is 46.29%. Our study is the first demonstration about the existence of the life cycles of four species of fish borne trematode infections around Tachileik, the Mekong region of Myanmar. The results of our study contribute important information for collaborative prevention of carcinogenic liver fluke infection among the lower Mekong region countries. In our findings of freshwater cyprinoid fishes from Tachileik, lower Mekong region of Myanmar, *Haplorchis pumilio* metacercariae have been collected in 5 species among the total 12 species i.e., *Barbonymus gonionotus, Puntioplites Falcifer, Mystacoleucus marginatus, Cyclocheilichthys repasson, Systomus Rubripinnis.* According to findings of (Chai et al 2017) and our new finding of *Haplorchis pumilio* metacercariae, it can be reported that total of thirteen species of fishes are the second intermediate host of *Haplorchis pumilio* metacercariae in Myanmar. The life cycles of *Haplorchis pumilio* are existing around Myanmar.

In Myanmar, seven species of minute intestinal flukes i.e., *H. taichui, H. pumilio, H. yokogawai, Centrocestus sp., Stellantchasmus falcatus, Pygidiopsis cambodiensis, and Procerovum sp*., were reported in fishes from local market of Yangon, Myanmar in 2017 by Chai et al 2017.They found that *H. taichui* metacercariae were detected in 5 species of fishes, *T. thynnoides, P. aurotaeniatus, E. altus, Mystacoleucus sp, Labeo sp*. In our present study, 4 out of 12 species of freshwater fishes were found positive for *H. taichui* metacercariae i.e., *M. marginatus, C. repasson, L. siamensis, R. argyrotaenia.* Therefore, a total of 9 species of freshwater fishes from Myanmar are confirmed to be second intermediate hosts of *H. taichui* metacercariae. Currently, *H. taichui metacercariae* have been identified as occurring in 48 species of fresh water cyprinoid fishes in southeast Asia countries. In addition, we found that *H. pumilio* metacercariae infected 5 out of 12 species i.e., *B. gonionotus, P. falcifer, M. marginatus, C. repasson, S. Rubripinnis.* In the study from J. Chai et al 2017, *H. pumilio* metacercariae were detected in 9 species of freshwater

fishes; *T. thynnoides, P. aurotaeniatus, Esomus altus, C. striata, A. testudineus, Rhynogobius sp., T. pectoralis, Mystacoleucus sp., Labeo sp.* Hence, a total of 14 species of freshwater fishes from Myanmar are the second intermediate hosts of *H. pumilio* metacercariae. Currently, a total of 39 freshwater cyprinoid fish species have been recorded as the second intermediate host of *H. pumilio* in southeast Asia countries. The metacercariae *Haplorchoides* sp. were infected in 5 species among the total 12 species i.e., *L. siamensis, H. siamensis, M. marginatus, R. argyrotaenia, C. repasson.* The finding of *Haplorchoides* sp. from our study is the first reported from Myanmar. In 2018, K. Apiwong et al reported finding *Haplorchoides* sp.in *B. schwanenfeldii, C. repasson* from Chiang Mai province, Thailand. Our study confirms that the life cycle of *Haplorchoides sp.* is existing around Tachileik, the Mekong Region of Myanmar.

In 2019, *Opisthorchis viverrini* metacercariae were found 4 species of fish forest snakehead (*Channa Lucius*), striped snakehead (*C. striata*), climbing perch (*Anabas testudineus*) and unspecified *Puntioplites sp* (Sohn et al 2019). They have reported that Low-Grade Endemicity of Opisthorchiasis, Yangon, Myanmar. They found that *Opisthorchis viverrini* metacercariae in freshwater fish and 0.7% fecal egg-positive rate of residents of Opisthorchis viverrini infection (Sohn et al 2019). In central Myanmar, Bago region Sanpool et al (2018) reported that Opisthorchis viverrini metacercariae were detected in in cyprinoid fish, Puntius brevis. (Sanpool et al., 2018). In Myanmar fish borne trematode infection is first reported in 2017 by (Chai et al 2017). Seven species of minute intestinal flukes, i.e., *Haplorchis taichui, H. pumilio, H. yokogawai, Centrocestus spp., Stellantchasmus falcatus, Pygidiopsis cambodiensis, and Procerovum sp.*, were found in fishes from local market of Yangon, Myanmar. *Haplorchis taichui* metacercariae were infected in 5 species *Thynnichthys thynnoides, Puntius aurotaeniatus, Esomus altus, Mystacoleucus sp, Labeo sp* (Chai et al., 2017).

In our study, from Bago, the Central region of Myanmar, we collected 125 fishes (4 species) and found 56 fishes (3 species) are contaminated 3 species metacercariae; Haplorchis taichui, Haplorchis pumilio, Haplorchoides sp. The overall prevalence is 44.8%. Bago is the located in Bago Region, Central region of Myanmar which is also an area of main source of production of raw small cyprinoid fishes

pickled with rice, locally called (Nyar lay Chin). Aung et al 2017 demonstrated, for the first time, *O. viverrini* from rural people in Myanmar. During stool surveys of rural populations in three regions of Lower Myanmar, Opisthorchis-like eggs were found in 34 out of 364 (9.3%) participants by stool microscopy after using the modified formalin-ether concentration technique. The overall prevalence of *Opisthorchis*-like eggs was 9.3% with the highest positive rate in the Bago Region (18.9%), followed by Mon State (5%) and Yangon Region (3.6%)Han et al 2019 reported that emerging neglected helminthiasis and determinants of multiple helminth infections in flood-prone township in Myanmar. A cross-sectional implementation research study was conducted in Shwegyin Township, Bago-East Region and they also found 17 cases of *O. viverrani/C. sinensis* (liver fluke) from Inland villages (n = 298) and 25 cases from Riverside villages (n = 400) (Han et al 2019). In our study showed the first occurrence about the existence of the life cycles 3 members of the Heterophyidae) around Bago, the Central region of Myanmar. This data will support for increasing public health awareness about the Fish borne trematode infections. Therefore, local people should be educated for the danger of ingestion of undercooked fish dishes and increased awareness for the prevention of fish borne trematode transmission.The Republic of the Union of Myanmar (Myanmar) is a sovereign state in the region of Southeast Asia, and bordered by India and Bangladesh to its west, Thailand and Lao PDR to its east, and China to its north and northeast.According to literature review, as the severe chronic infection of *O. viverrini* is a strong risk factor for cholangiocarcinoma (Honjo et al., 2005) (Haswell-Elkins et al., 1994). The highest prevalence of infection in secondary intermediate host occur in North and Northeast Thailand, especially in rural populations (Sripa et al., 2011) (Jongsuksuntigul et al., 2003)(Sithithaworn et al., 2012) and in the adjacent southern and central regions of Lao People's Democratic Republic (Forrer et al., 2012; Sayasone et al., 2009).

Human infections with *O. viverrini* and presence of metacercariae in intermediate hosts have been reported in several provinces of Cambodia (Chai et al., 2014) (Miyamoto et al., 2014) (Sohn et al., 2012) (Yong et al., 2014). Miyamoto et al reported O. viverrini eggs in human fecal samples from 26 out of 55 surveyed villages in five provinces of Cambodia, among which 15 villages had an egg positive rate >10% (Miyamoto et al., 2014). The parasite is also endemic in southern and central

parts of Vietnam (Dao et al., 2016). (Dung et al., 2014). A survey conducted in 2015 reported that the overall prevalence of *O. viverrini* infection was 11.4% in central Vietnam (Dao et al., 2016). Although not many reported to date from Myanmar, the presence of *O. viverrini* is likely because of its close proximity to endemic areas in Thailand and because of an open-borders policy that started in 2015 leading to increasing migration among ASEAN Economic Community (AEC) countries (Thailand, Lao People's Democratic Republic, Cambodia, Vietnam and Myanmar) (ASEAN Economic Community Blueprint. Declaration on the ASEAN Economic Community blueprint 2008. http://asean.org/wp-content/uploads/archive/51 87-10.pdf Accessed 4Mar 2017) .Approximately eight million people in Thailand and two million in Lao PDR are estimated to be infected with *O. viverrini* (Sripa et al., 2010) with high prevalence reported in rural populations of North and Northeast Thailand (Sripa et al., 2011; Jongsuksuntigul and Imsomboon,, 2003; Sithithaworn et al., 2012) and in the adjacent central and southern parts of Lao PDR (Forrer et al., 2012; Sayasone et al., 2009). In Thailand, the epicenter of *Opisthorchis viverrini* infection is located in north and northeast Thailand, where high a prevalence of opisthorchiasis coexists with a high incidence of cholangiocarcinoma (CHCA). Southeast Asian liver fluke (*Opisthorchis viverrini*) and Chinese liver fluke (*Clonorchis sinensis*) are classified as Group 1 carcinogens, i.e. they are substantiated and directly cancer-causing agents (Bouvard et al., 2009). *O. viverrini* is a food-borne liver fluke that mainly attacks the area of the bile duct. Infection with the parasite, called opisthorchiasis is the major cause of cholangiocarcinoma, a cancer of the bile ducts, in northern Thailand, the Lao People's Democratic Republic, Vietnam and Cambodia. (Do et al., 2007; 'Saenphet et al., 2008; Lovis et al., 2009; Sithithaworn et al., 2003; Chai et al., 2017; Wattanayingcharoenchai et al., 2011; Jeon et al., 2012).

We studied two locations from north of Thailand ie; Chom Thong district and Mae Ngat reservoir in Chiang Mai Province. Total of 530 fishes (8 different species of cyprinoids fishes) were collected from local market Chiang Mai Province, North of Thailand and total of 318 fishes (5 different species of cyprinoids fishes) were collected Mae Ngat reservoir, Chiang Mai, North of Thailand. Four locations from northeast of Thailand ie; NikhomKham Soi, Sakon Nakhon, Sisaket, Kalasin. From Northeast of Thailand, total of 335 fishes (5 different species of cyprinoids

fishes) were collected from Nikom KomeSoi, Northeast of Thailand. Total of 285 fishes (3different species of cyprinoids fishes) were collected from Sakon Nakhon, Northeast of Thailand. Total of 168 fishes (6 different species of cyprinoids fishes) were collected from Sisaket, Northeast, Thailand. Total of 434 fishes (5 different species of cyprinoids fishes) were collected from Kalasin, Northeast of Thailand.In our study from Mae Ngat reservoir, Chiang Mai, Thailand, we collected 318 fishes (5 species) and found 129 fishes (3 species) are infected with 2 species metacercariae *Haplorchis taichui, Haplorchoides* sp. The overall prevalence is 40.56%. Our new finding from Chom Thong District, Chiang Mai, Thailand, we collected 530 fishes (8 species) and occurred 284 fishes (4 species) are contaminated with 4 species metacercariae; *Opisthorchis viverrini, Haplorchis taichui, Haplorchis pumilio, Haplorchoides* sp. The overall prevalence is 53.58%. The new occurrence of FZT. Boonmekam et al 2016 demonstrated the prevalence of human intestinal fluke infections, Haplorchis taichui, in thiarid snails and cyprinid fish in Bo Kluea District and Pua District, Nan Province, Thailand. They found in the 10 species of cyprinid fish (i.e; *Barbodes schwanenfeldi, Garra cambodgiensis, Hypsibarbus salweenensis, Mystacoleucus marginatus, Osparus pulchellus, Poropuntius deauratus, P. normani, Scaphiodonicthys acanthopterus, Systomus orphoides* and *S. stolitzkaenus),* metacercariae were found in all of them. Among them, *B. schwanenfeldi, M. marginatus, P. deauratus, P. normani and S. orphoides* had very high infection rates. Morphometric confimed those metacercariae to represent the heterophyid, *Haplorchis taichui* (Boonmekam et al., 2016). (Nithikathkul C, 2008). reported about the prevalence of *Haplorchis taichui* and *Haplorchoides* sp. Metacercariae in Freshwater Fish from Water Reservoirs, Chiang Mai, Thailand They studied that parasitological investigation on trematode metacercariae was made on 62 freshwater fishes of 13 species in northern Thailand; *Cyclocheilichthys apogon, Puntioplites proctozysron, Labiobarbus siamensis, Barbodes gonionotus, Barbodes altus, Henicorhynchus siamensis, Osteochilus hasselti, Notopterus notopterus, Mystacoleucus marginatus, Anabas testudineus, Systomus orphoides, Morulius chrysophykadian, and Hampala macrolepidota* from the Mae Ngad and the Mae Kuang Udomtara Reservoirs in Chiang Mai province, Thailand. The highest intensity of heterophyid metacercariae in *H. siamensis* in the Mae Ngad was 120.4 and that in *P. proctozysron* in the Mae

Kuang Udomtara was 180.0. The fish, *A. testudineus*, *C. apogon*, and *M. chrysophykadian*, were not found to be infected with *H. taichui* metacercariae. The results show that the freshwater fish in Chiang Mai water reservoirs are heavily infected with *H. taichui* and *Haplorchoides* sp. metacercariae (Nithikathkul et al 2008). Sukontason et al 2001 investigated the intensity of infection of trematode metacercariae was determined in five species of cyprinoid fish collected from Mae Ngud reservoir, Chiang Mai Province. These species were *Thynnichthys thynnoides, Puntioplites proctozysron, Hampala macrolepidota, Puntius leiacanthus* and *Puntius gonionotus. T. thynnoides* contained the highest number (83.0%) of metacercariae, whereas *P. gonionotus* had the fewest (0.19%). Most metacercariae was isolated from *Haplorchis taichui* (63.27%), with an intensity variation of 0.3- 165.2 metacercariae/fish (Sukontason et al 2001). Sukontason et al 1999 reported that Prevalence of trematode metacercariae from cyprinoid fish of Ban Pao district, Chiang Mai Province, northern Thailand and their results showed *Opisthorchis viverrini* (0.2%), minute intestinal flukes *Haplorchis taichui* (87.0%), *Haplorchis pumilio* (3.2%) (Sukontason et al., 1999).

A previous study had investigated the intensity of infection of trematode metacercariae was determined in five species of cyprinoid fish collected from Mae Ngud reservoir, Chiang Mai Province. These species were *Thynnichthys thynnoides, Puntioplites proctozysron, Hampala macrolepidota, Puntius leiacanthus* and *Puntius gonionotus. T. thynnoides* contained the highest number (83.0%) of metacercariae, whereas *P. gonionotus* had the fewest (0.19%). Most metacercariae was isolated from *Haplorchis taichui* (63.27%), with an intensity variation of 0.3- 165.2 metacercariae/fish. (Sukontason et al., 2001). In 2013, Wongsawad et al found Five species of metacercariae from Fang-Mae Ai Agricultural Basin, Chiang Mai province, Thailand. There was one species of liver fluke, *Opisthorchis viverrini*, and four species of minute intestinal flukes, *viz. Haplorchis taichui*, *Haplorchoides* sp., *Centrocestus caninus*, and *Stellantchasmus falcatus*. The prevalence of trematodes were *Haplorchoides* sp. (37.43%), *H. taichui* (35.66%), *C. caninus* (3.80%), *S. falcatus* (1.40%), and *O. viverrini* (0.44%) (Wongsawad et al 2013). *Haplorchis taichui* infections are commonly found in the northern and northeastern regions of Thailand. Several species of cyprinoid fish have been reported as the second

intermediate hosts (Srisawangwong et al., 1997) (Namue et al., 1998) (Wongsawad et al., 2000). Chom Thong district showed the highest prevalence and intensity of metacercarial infection in fish. It is possible that this area is one of the high-risk areas in Chiang Mai Province. In addition, Thai traditional dishes, such as 'blah-sohm' and 'lahp-blah', are believed to be a sources of infection in people. These raw-fish dishes cannot induce degeneration of the contaminated metacercariae in a short period (Wiwanitkit et al, 2003), and there is a tendency that the number of worms infecting humans will increase because of acquiring the infection from eating raw-fish dishes.

In our research study from Nikhom Kham Soi, Northeast of Thailand, in the total of 335 fishes, 185 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 55.22 %. Among the 5 different species, 2 species of cyprinoid fishes, i.e., *Barbonymus gonionotus*, *Balantiocheilos ambusticauda* were contaminated with the infective stage of trematode parasites, metacercariae The metacercariae of *O. viverrini* were found in *Balantiocheilos ambusticauda* .The metacercariae of *H. taichui* were found in *Barbonymus gonionotus* .The metacercariae of *H. pumilio* were infected in *Balantiocheilos ambusticauda* The metacercariae *Haplorchoides* sp. were present in 2 out of 5 species of fishes, *Barbonymus gonionotus* and *Balantiocheilos ambusticauda.* From Sakon Nakhon, Northeast of Thailand, in the total of 285 fishes, 165 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 57.89%. Among the 3 different species, 2 species of cyprinoid fishes, i.e., *Mystacoleucus marginatus*,*Henicorhychus siamemsis* were contaminated with the infective stage of trematode parasites, metacercariae. The metacercariae of *O. viverrini* were found in *Mystacoleucus marginatus.* The metacercariae of *H. taichui* were found infecting *Mystacoleucus marginatus, Henicorhychus siamemsis* species of fishes among the total 3 species of fishes. The prevalence of infection was found in *Mystacoleucus marginatus* and *Henicorhychus siamemsis*. The metacercariae of *H. pumilio* were infected in *Henicorhychus siamemsis.* The metacercariae *Haplorchoides* sp. were present in in *Mystacoleucus marginatus* and *Henicorhychus siamemsis.* From Sisaket, Northeast of Thailand we collected 168 fishes (6 species) and found 46 fishes (1 species) are contaminated with *Haplorchoides sp.* The overall prevalence is 27.38%. From Kalasin, Northeast of Thailand, we collected 434 fishes (5 species) and found 175

fishes (3 species) are contaminated *Haplorchoides sp.* The overall prevalence is 40.32%.

Prakobwong et al 2017 reported an integrated epidemiological study of *Opisthorchis viverrini* consisting of risk factors analysis and parasitation prevalence determination in humans, as well as the assessment the roles of host reservoirs (dogs and cats) and intermediate hosts (cyprinid fish) was carried out in the vicinity of Huay Luang dam, Udon Thani province, in the north east of Thailand. he incidence of *O. viverrini* metacercariae in four cyprinid fi shes *Barbonymus gonionotus* (n = 124), *Cyclocheilichthys repasson* (n = 843), *Hampala dispar* (n = 276) and *Henicorhynchus siamensis* n = 946), were determined and overall values ranged from 2.4 % to 23.1 %. There was a seasonal variation in metacercariae intensity which ranged from 1–125metacercariae per fish (Prakobwong et al 2017). Pinlasor et al 2013 conducted a cross-sectional survey on 12,890 fish consisting of 13 species randomly caught from 26 rivers, 10 dams, and 38 ponds/lakes from 20 provinces in northeastern Thailand. *Opisthorchis viverrini* metacercariae were found in 6 species of fish; i.e., *Cyclocheilichthys armatus, Puntius orphoides, Hampala dispar*, *Henicorhynchus siamensis, Osteochilus hasselti, and Puntioplites proctozysron* from localities in 13 provinces. The mean intensity of *Opisthorchis viverrini* metacercariae ranged from 0.01 to 6.5 cysts per fish (or 1.3-287.5 cysts per kg of fish). A high mean intensity of *Opisthorchis viverrini* metacercariae per fish (>3 cysts) was found in 5 provinces: Amnat Charoen (6.5 cysts), Nakhon Phanom (4.3), Mukdahan (4.1), Khon Kaen, (3.5) and Si Sa Ket (3.4). In conclusion, OV MC are prevalent in natural cyprinid fish, with the infection rate varying according to fish species and habitats (Pinlasor et al 2013). Onsurathum et al 2016 surveyed Fish borne trematode metacercariae in fermented fish dishes (pla-ra and plasom) obtained from 73 local markets in 20 provinces of northeastern Thailand. Fish borne trematode metacercariae were detected in fermented fish dishes from markets located in five provinces: Si Sa Ket, Sakon Nakhon, Mukdahan, Khon Kaen and Udon Thani.Fish borne trematode metacercariae were found in four species of fish: *Henicorhynchus siamensis, Puntius bimaculatus, Puntius orphoides and Hampala dispar*. (Onsurathum et al., 2016). In our present study from Nikhom kham soi, Thailand we collected 335 fishes (5 species) and occurred 185 fishes (2 species) are infected with 4 species metacercariae.

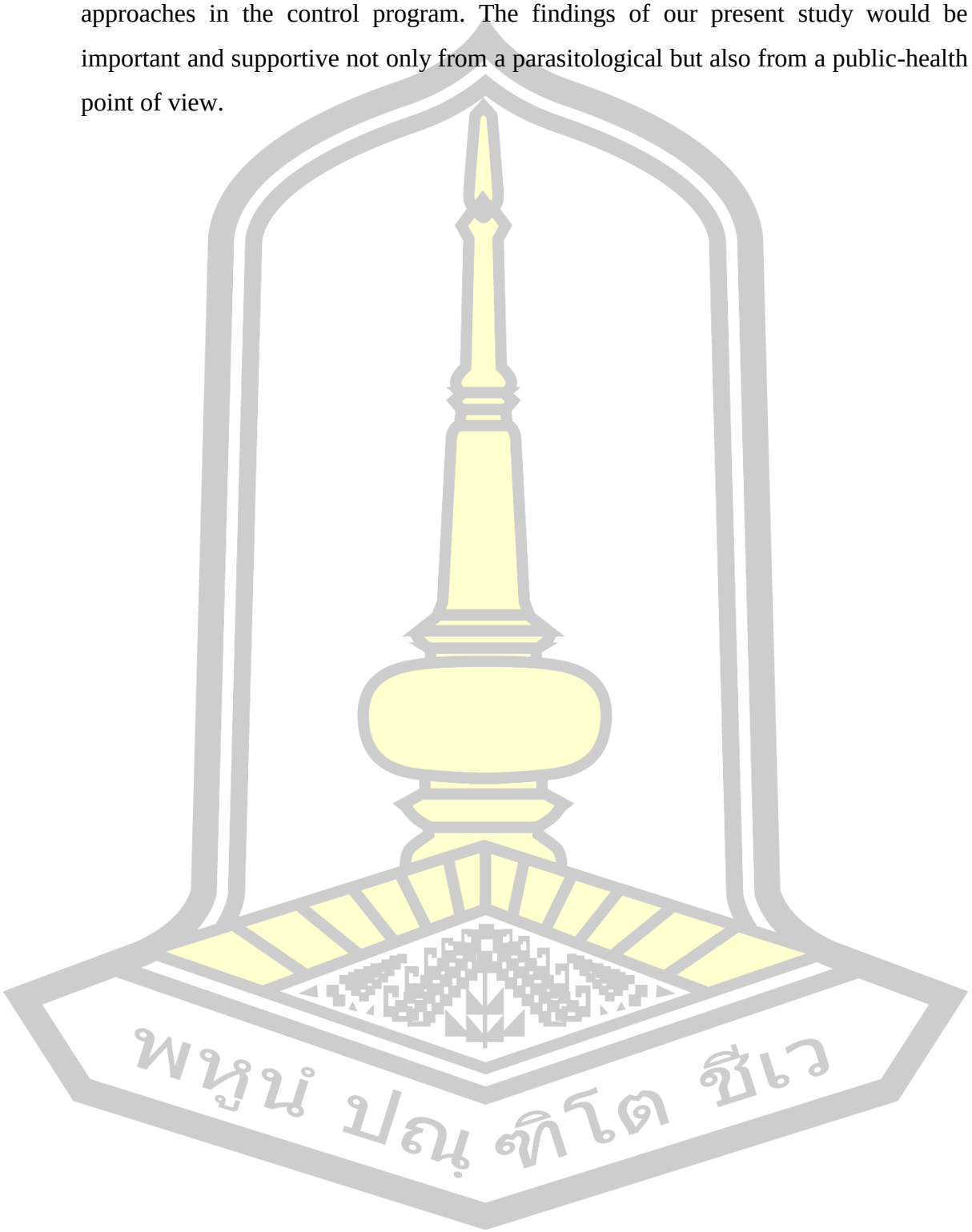
Opisthorchis viverrini, Haplorchis taichui, Haplorchis pumilio, Haplorchoides sp. The overall prevalence is 55.23%. From Sakon Nakhon, Thailand, we collected 285 fishes (3 species) and found 165 fishes (2 species) are contaminated with 4 species metacercariae; *Opisthorchis viverrini, Haplorchis taichui, Haplorchis pumilio, Haplorchoides* sp. The overall prevalence is 57.89 %. Consumption of raw or inadequately cooked cyprinid fish as well as related products (often contaminated with *Opisthorchis viverrini and/or Haplorchis taichui*) is one of the major causes of fish-borne trematode infection, which is still endemic in the Greater Mekong Subregion; Vietnam, Laos, Myanmar, Cambodia and Thailand (Chai et al., 2005). Chronic infection caused by the Southeast Asian liver fluke (*O. viverrini*) is a critical risk factor for the development of the bile duct cancer cholangiocarcinoma (CCA), which is a major public health concern in Mekong region countries. *O. viverrini* infection in humans occurs via the consumption of a raw or uncooked fish which contains metacercariae. A better understanding of the epidemiology of this fish borne parasites are important to investigate for the prevention of CCA in the community.

A GIS database for the study of fish-borne metacercariae implemented using an ArcGIS Desktop program from the ESRI Company, Bangkok, Thailand. A geographic information system (GIS) is a framework for gathering and analyzing data. GIS integrates many types of data. It analyzes spatial location and organizes layers of information into visualizations using maps and 3D scenes. With this unique capability, GIS reveals deeper insights into data, by helping users make smarter decisions. In our study, we investigated environmental factors that influence to fish-borne metacercariae, the GIS database was overlaid with population density and precipitation GIS datasets. Geographic coordinates of each area are determined with a Global Positioning System. Maps are the geographic container for the data layers. GIS maps are easily shared and embedded in apps and accessible by virtually everyone, everywhere. GIS integrate many different kinds of data layers using spatial location. Most data have a geographic component. GIS data includes imagery, features and base maps linked to spreadsheets and tables. Spatial analysis lets you evaluate suitability and capability, estimate and predict, interpret and understand and much more, lending new perspectives to insight and decision-making. Apps provide focused user experiences for getting work done and bringing GIS to life for everyone. GIS

apps work virtually everywhere: on your mobile phones, tablets, in web browsers and on desktops. The geographic information (latitude and longitude) associated with the infection rates among susceptible species of fresh water fish was recorded and built a geo-dataset for GIS development. The GIS could be useful in the establishment of prevention strategies for transmission of food borne diseases from infected fish in the water catchment area. The distribution of fish-borne trematode infections is highly focal, dependent on the presence of susceptible second intermediate hosts, the prevalence of fish-borne parasitic infections in the definitive host and behavioral patterns of the definitive hosts. The infections are endemic in areas where raw fish eating habits are deeply rooted in the culture and are difficult to change. The Ministry of Public Health should encourage intense targeted educational programs for high risk populations to discourage the consumption of raw fish. Further study on the correlation of clinical symptoms with environmental and geographic information may offer a comprehensive strategy to the helminthes dilemma.

In conclusion, four species of trematode metacercariae (carcinogenic liver flukes, *O.viverrini* and minute intestinal flukes, members of the Heterophyidae, *H. taichui*, *H. pumilio*, *Haplorchoides* sp.) were detected in 8 locations of study area . Our study demonstrates the existence of the life cycles of four species of fish borne trematode infections around Tachileik, the Mekong region of Myanmar. Moreover, our findings showed the first occurrence about the existence of the life cycles 3 members of the Heterophyidae) around Bago, the Central region of Myanmar. This data will support for increasing public health awareness about the Fish borne trematode infections. The study of the distribution and infection status of the fish-borne trematode metacercariae could contribute to solving the important public health problem posed by these trematodes and may provide valuable information for prevention and control programs of human liver fluke and intestinal fluke infections for the community. Furthermore, the outcome of this study could be a useful index in the trematode epidemiology in Mekong area. In addition, this study might provide evidence leading to improved public health awareness for surveillance and control of FBT contamination. In addition, the results of our study contribute important information for collaborative prevention of carcinogenic liver fluke infection among the lower Mekong region countries. Further research studies should be considered for

public health interventions using health-education and sanitation-improvement approaches in the control program. The findings of our present study would be important and supportive not only from a parasitological but also from a public-health point of view.



REFERENCES

References

Anh NT, Phuong NT, Johansen MV, Murrell KD, Van PT, Dalsgaard A, Thu LT, T. S. (2009). Prevalence and risks for fishborne zoonotic trematode infections in domestic animals in a highly endemic area of North Vietnam. *Acta Tropica*, 112(2), 198-203.

Anh NT, Phuong NT, Murrell KD, Johansen MV, Dalsgaard A, Thu LT, Chi TT, T. S. (2009). Animal reservoir hosts and fish-borne zoonotic trematode infections on fish farms, Vietnam. *Emerging infectious diseases,*15(4), 540.

Aung WP, Htoon TT, Tin HH, Thinn KK, Sanpool O, Jongthawin J, Sadaow L, Phosuk I, Rodpai R, Intapan PM, M. W. (2017). First report and molecular identification of Opisthorchis viverrini infection in human communities from Lower Myanmar. *Plo S one,*12(5), e0177130.

Boonmekam D, Namchote S, Glaubrecht M, K. D. (2016). The prevalence of human intestinal fluke infections, Haplorchis taichui, in thiarid snails and cyprinid fish in Bo Kluea District and Pua District, Nan Province, Thailand. *Scicence, Engineering and Health Studies.* 10(3), 29-37.

Boonmekam, D., Namchote, S., Matsuda, H., Kirinoki, M., Miyamoto, K., Sinuon, M., & Krailas, D. (2017). Morphological and molecular identification of the liver fluke Opisthorchis viverrini in the first intermediate host Bithynia snails and its prevalence in Kampong Cham Province, Cambodia. *Parasitology International*, 66(3), 319–323. https://doi.org/10.1016/j.parint.2017.01.016

Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, C. V. (2009). A review of human carcinogens--Part B: biological agents. *The Lancet. Oncology*, 10(4), 321.

Callahan MP. (2017). Distorted, dangerous data. Lumyo in the 2014 Myanmar population and housing census. *Sojourn: Journal of Social Issues in Southeast Asia*, 32(2), 452-78.

Chai, J., Sohn, W., Na, B., Park, J., Jeoung, H., & Hoang, E. (2017). Zoonotic Trematode Metacercariae in Fish from Yangon. *Myanmar and Their Adults Recovered from Experimental Animals*, *55*(6), 631–641.

Chai, J. Y., Sohn, W. M., Na, B. K., Park, J. B., Jeoung, H. G., Hoang, E. H., … Tin, H. H. (2017). Zoonotic trematode metacercariae in fish from yangon, Myanmar and their adults recovered from experimental animals. *Korean Journal of Parasitology*, 55(6), 631–641. https://doi.org/10.3347/kjp.2017.55.6.631

Chai, J. Y., Sohn, W. M., Na, B. K., Yong, T. S., Eom, K. S., Yoon, C. H., … Socheat, D. (2014). Zoonotic Trematode metacercariae in fish from Phnom Penh and Pursat, Cambodia. *Korean Journal of Parasitology*, *52*(1), 35–40. https://doi.org/10.3347/kjp.2014.52.1.35

Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY,Um JY, Park MS, Hoang EH, Phommasack B, Insisienmay B, Lee SH, R. H. (2009).. High prevalence of Haplorchis taichui, Phaneropsolus molenkampi, and other helminth infections among people in Khammouane Province, Lao PDR *Korean J Parasitol* , 47, 243-247.

Chai JY, Murrell KD, F. B. (2007). Food-borne parasitic zoonoses: Fish and plant-borne parasites. World Class Parasites. *Intestinal flukes*, 11, 53-115.

Chai JY, Park JH, Han ET, Guk SM, Shin EH, Lin A, Kim JL, Sohn WM, Yong TS, Eom KS, Min DY, Hoang EH, Phommasack B, Insisienmay B, R. H. (2005). Mixed infections with Opisthorchis viverrini and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos. *J Helminthol,* 79, 283-289.

Chai JY, Sohn WM, Na BK, Park JB, Jeoung HG, Hoang EH, Htoon TT, T. H. (2017). Zoonotic trematode metacercariae in fish from Yangon, Myanmar and their adults recovered from experimental animals. *The Korean journal of parasitology*, 55(6), 631.

Chai JY, Sohn WM, Na BK, Yong TS, Eom KS, Yoon CH, Hoang EH, Jeoung HG, S. D. (2014). Zoonotic trematode metacercariae in fish from Phnom Penh and Pursat, Cambodia. *The Korean journal of parasitology*, 52(1), 35.

Dao HT, Dermauw V, Gabriël S, Suwannatrai A, Tesana S, Nguyen GT, D. P. (2017). Opisthorchis viverrini infection in the snail and fish intermediate hosts in Central Vietnam. *Acta tropica*, 170, 120-5.

Dao TT, Van Bui T, Abatih EN, Gabriël S, Nguyen TT, Huynh QH, Van Nguyen C, D. P. (2016). Opisthorchis viverrini infections and associated risk factors in a lowland area of Binh Dinh Province, Central Vietnam. *Acta tropica*, 157, 151-157.

Do, T. D., Van De, N., Waikagul, J., Dalsgaard, A., Chai, J. Y., Sohn, W. M., & Murrell, K. D. (2007). Fishborne zoonotic intestinal trematodes, Vietnam. *Emerging Infectious Diseases*, 13(12), 1828–1833.

Do Trung Dung NV, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, M. K. (2007). Fishborne zoonotic intestinal trematodes, Vietnam. *Emerging Infectious Diseases*, 13(12), 1828.

Dorny P, Praet N, Deckers N, G. S. (2009). Emerging food-borne parasites. *Veterinary parasitology*, 163(3),196-206.

Dung DT, De NV, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, M. K. (2007). Fishborne intestinal zoonotic trematodiasis, Vietnam. *Emerg Inf Dis*, 13, 1828-1833.

Dung VT, Waikagul J, Thanh BN, Vo DT, Nguyen DN, M. K. (2014). Endemicity of Opisthorchis viverrini liver flukes, Vietnam, 2011–2012. *Emerging infectious diseases*, 20(1), 152.

Dusit Boonmekam , Suluck Namchote , Worayuth Nak-ai, M. G. and D. K. (2016). The Prevalence of Human Intestinal Fluke Infections , Haplorchis taichui , in Thiarid Snails and Cyprinid Fish in Bo Kluea District and Pua District , 10(3), 29–37. https://doi.org/10.14456/sustj.2016.10

Eom, K. S., Park, H. S., Lee, D., Sohn, W. M., Yong, T. S., Chai, J. Y., … Phommasack, B. (2015). Infection status of zoonotic trematode metacercariae in fishes from Vientiane municipality and Champasak Province in Lao PDR. *Korean Journal of Parasitology*, *53*(4), 447–453. https://doi.org/10.3347/kjp.2015.53.4.447

Forrer A, Sayasone S, Vounatsou P, Vonghachack Y, Bouakhasith D, Vogt S, Glaser R, Utzinger J, Akkhavong K, O. P. (2012). Spatial distribution of, and risk factors for, Opisthorchis viverrini infection in southern Lao PDR. *PLoS Negl Trop Dis*, 6(2), e1481.

Green A, Uttaravichien TH, Bhudhisawasdi VA, Chartbanchachai WI, Elkins DB, Marieng EO, Pairqjkul C, Dhiensiri TU, Kanteekaew NA, H.-E. M. (1991). Cholangiocarcinoma in north east Thailand. A hospital-based study. *Tropical and geographical medicine*, 43(1-2), 193.

Hahmann S, B. D. (2013). How much information is geospatially referenced? Networks and cognition. *International Journal of Geographical Information Science*, 27(6), 1171-1189.

Haswell-Elkins MR, Satarug S, Tsuda M, Mairiang E, Esumi H, Sithithaworn P, Mairiang P, Saitoh M, Yongvanit P, E. D. (1994). Liver fluke infection and cholangiocarcinoma: model of endogenous nitric oxide and extragastric nitrosation in human carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 305(2),241-52.

Hitanant S, Trong DT, Damrongsak C, Chinapak O, Boonyapisit S, Plengvanit U, V. V. (1987). Peritoneoscopic findings in 203 patients with Opisthorchis viverrini infection. *Gastrointestinal endoscopy*, 33(1), 18-20.

Honjo S, Srivatanakul P, Sriplung H, Kikukawa H, Hanai S, Uchida K, Todoroki T, Jedpiyawongse A, Kittiwatanachot P, Sripa B, D. S. (2005). Genetic and environmental determinants of risk for cholangiocarcinoma via Opisthorchis viverrini in a densely infested area in Nakhon Phanom, northeast Thailand. *International Journal of Cancer*, 117(5), 854-60.

Hung N, Madsen H, F. B. (2013). Global status of fish-borne zoonotic trematodiasis in humans. *Acta Parasitologica*, 58(3), 231-58.

Jeon HK, Lee D, Park H, Min DY, Rim HJ, Zhang H, Yang Y, Li X, E. K. (2012). Human infections with liver and minute intestinal flukes in Guangxi, China: analysis by DNA sequencing, ultrasonography, and immunoaffinity chromatography. *The Korean journal of parasitology*, 50(4), 391.

Jongsuksuntigul P, and Imsomboon T. (2003). Opisthorchiasis control in Thailand. *Acta tropica*, 88(3), 229-232.

Jongsuksuntigul P, I. T. (2003). Opisthorchiasis control in Thailand. *Acta tropica.* 88(3), 229-32.

Keiser J, U. J. (2009). Food-borne trematodiases. *Clinical microbiology reviews,* 22(3), 466-83.

King S, and S. T. (2001). ***Trematodes of the family Opisthorchiidae: a minireview.*** *The Korean journal of parasitology,* 39(3), 209.

Krailas D, Veeravechsukij N, Chuanprasit C, Boonmekam D, N. S. (2016). Prevalence of fish-borne trematodes of the family Heterophyidae at Pasak Cholasid Reservoir, Thailand. *Acta tropica*, 156, 79-86.

Kyaw PP, Than CP, Thet TM, M. T. (2019). Changing trend of Cholangiocarcinoma in 4 Years (Myanmar). *HPB*. 21, S466.

Landis SH, Murray T, Bolden S, W. P. (1998). Cancer statistics, 1998. *CA: a cancer journal for clinicians,* 48(1), 6-29.

Lima dos Santos CA, H. P. (2011). Fishborne zoonotic parasites and aquaculture: a review. *Aquaculture*, 318(3-4), 253-61.

Lovis L, Mak TK, Phongluxa K, Soukhathammavong P, Sayasone S, Akkhavong K, Odermatt P, Keiser J, F. I. (2009). PCR diagnosis of Opisthorchis viverrini and Haplorchis taichui infections in a Lao community in an area of endemicity and comparison of diagnostic methods for parasitological field surveys. *Journal of Clinical Microbiology*, 47(5), 1517-23.

Miyamoto K, Kirinoki M, Matsuda H, Hayashi N, Chigusa Y, Sinuon M, Chuor CM, K. V. (2014). Field survey focused on Opisthorchis viverrini infection in five provinces of Cambodia. *Parasitology international*,63(2), 366-73.

Namue C, Rojanapaibul A, W. C. (1998). Occurrence of two heterophyid metacercariae Haplorchis and Haplorchoides in cyprinoid fish of some districts in Chiang Mai and Lumphun Province. *The Southeast Asian journal of tropical medicine and public health, 29(2), 401-5.*

Nithikathkul C, W. C. (2008). Prevalence of Haplorchis taichui and Haplorchoides sp. metacercariae in freshwater fish from water reservoirs, Chiang Mai, Thailand. *The Korean Journal of Parasitology*, 46(2), 109.

Onsurathum S, Pinlaor P, Charoensuk L, Haonon O, Chaidee A, Intuyod K, Laummaunwai P, Boonmars T, Kaewkes W, P. S. (2016). Contamination of Opisthorchis viverrini and Haplorchis taichui metacercariae in fermented fish products in northeastern Thailand markets. *Food Control*, 59, 493-8.

Onsurathum, S., Pinlaor, P., Charoensuk, L., Haonon, O., Chaidee, A., Intuyod, K., … Pinlaor, S. (2016). Contamination of Opisthorchis viverrini and Haplorchis taichui metacercariae in fermented fish products in northeastern Thailand markets. *Food Control*, 59, 493–498.

Pairojkul C, Shirai T, Hirohashi S, Thamavit W, Bhudhisawat W, Uttaravicien T, Itoh M, I. N. (1991). Multistage carcinogenesis of liver-fluke-associated cholangiocarcinoma in Thailand. *InPrincess Takamatsu Symposia*, 22, 77-86.

Phan VT, Ersbøll1 AK, Nguyen KV, Madsen H, D. A. (2010). Farm-level risk factors for fishborne zoonotic trematode infection in integrated small-scale fish farms in northern Vietnam. *PLoS Negl Trop Dis*, 4, e742.

Pinlaor, S., Onsurathum, S., Boonmars, T., Pinlaor, P., Hongsrichan, N., Chaidee, A., … Sithithaworn, P. (2013). Distribution and abundance of opisthorchis viverrini metacercariae in cyprinid fish in northeastern Thailand. *Korean Journal of Parasitology*, 51(6), 703–710. https://doi.org/10.3347/kjp.2013.51.6.703

Prakobwong S, Gunnula W, Chaipibool S, Nimala B, Sangthopo J, Sirivetthumrong N, R. A. (2017). Epidemiology of Opisthorchis viverrini in an endemic area of Thailand, an integrative approach. *Helminthologia,* 54(4), 298-306.

Prakobwong, S., Suwannatrai, A., Sancomerang, A., Chaipibool, S., & Siriwechtumrong, N. (2017). A Large Scale Study of the Epidemiology and Risk Factors for the Carcinogenic Liver Fluke Opisthorchis viverrini in Udon Thani Province, Thailand. *Asian Pacific Journal of Cancer Prevention : APJCP*, 18(10), 2853–2860. https://doi.org/10.22034/APJCP.2017.18.10.2853

Pumidonming W, Katahira H, Igarashi M, Salman D, Abdelbaset AE, S. K. (2018). Potential risk of a liver fluke Opisthorchis viverrini infection brought by immigrants from prevalent areas: A case study in the lower Northern Thailand. *Acta tropica,* 178, 213-8.

Pyo KH, Kang EY, Hwang YS, Jun HC, Sohn WM, Cho SH, Lee WJ, Chai JY, S. E. (2013). Species identification of medically important trematodes in aquatic food samples using PCR-RFLP targeting 18S rRNA. *Foodborne pathogens and disease*, 10(3), 290-2.

Radomyos B, Wongsaroy T, Wilairatana P, Radomyos P, Praevanich R, Meesomboon V, J. P. (1998). Opisthorchiasis and intestinal fluke infections in northern Thailand. *SoutheastAsian J Trop Med Public Health* , 29, 123-127.

RE., W. (1987). Selling a geographical information system to government policy makers. *In URISA* , 3, 150-6.

Rim, H. J., Sohn, W. M., Yong, T. S., Eom, K. S., Chai, J. Y., Min, D. Y., … Insisengmay, S. (2008). Fishborne trematode metacercariae detected in freshwater fish from Vientiane Municipality and Savannakhet Province, Lao PDR. *Korean Journal of Parasitology*, 46(4), 253–260. https://doi.org/10.3347/kjp.2008.46.4.253

Rim, H., Sohn, W., Yong, T., Eom, K. S., Chai, J., Min, D., … Insisengmay, S. (2008). Fishborne Trematode Metacercariae Detected in Freshwater Fish from Vientiane Municipality and Savannakhet Province . *Lao PDR*, 46(4), 253–260. https://doi.org/10.3347/kjp.2008.46.4.253

Ruenwongsa, P., Hutadilok, N., & Yuthavong, Y. (1983). Opisthorchis viverrini, *32*(c), 2529–2534.

Saenphet S, Wongsawad C, Saenphet K, Rojanapaibul A, Vanittanakom P, C. J. (2008). *The occurrence of heterophyid metacercariae in cyprinoid fish in Chiang Mai province. Southeast Asian journal of tropical medicine and public health*, 39(1), 56.

Saenphet, S., Wongsawad, C., Saenphet, K., Rojanapaibul, A., Vanittanakom, P., Chai, J., … Diseases, E. (2008). *the Occurrence of Heterophyid Metacercariae in*, 39(suppl 1).

Sanpool O, Aung WP, Rodpai R, Maleewong W, I. P. (2018). Human liver fluke Opisthorchis viverrini (Trematoda, Opisthorchiidae) in Central Myanmar: New records of adults and metacercariae identified by morphology and molecular analysis. *Acta tropica*. 185, 149-55.

Sanpool, O., Aung, W. P. P., Rodpai, R., Maleewong, W., & Intapan, P. M. (2018). Human liver fluke Opisthorchis viverrini (Trematoda, Opisthorchiidae) in Central Myanmar: New records of adults and metacercariae identified by morphology and molecular analysis. *Acta Tropica*, 185(May), 149–155. https://doi.org/10.1016/j.actatropica.2018.05.009

Sayasone S, Vonghajack Y, Vanmany M, Rasphone O, Tesana S, Utzinger J, Akkhavong K, O. P. (2009). Diversity of human intestinal helminthiasis in Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene*,103(3), 247-54.

Scholz T, Ditrich O, G. M. (2019). Differential diagnosis of opisthorchiid and heterophyid metacercariae (Trematoda) infecting flesh of cyprinid fish from Nam Ngum Dam Lake in Laos. *The Southeast Asian journal of tropical medicine and public health*, 22, 171-3.

Shin HR, Oh JK, Masuyer E, Curado MP, Bouvard V, Fang YY, Wiangnon S, Sripa B, H. ST. (2010). Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer science*. 101(3), 579-85.

Sithithaworn P, Andrews RH, Van De N, Wongsaroj T, Sinuon M, Odermatt P, Nawa Y, Liang S, Brindley PJ, S. B. (2012). The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitology international*, 61(1), 10-6.

Sithithaworn P, and H.-E. M. (2003). Epidemiology of Opisthorchis viverrini. *Acta tropica*, 88(3), 187-94.

Sohn, W. M. (2009). Fish-borne zoonotic trematode metacercariae in the Republic of Korea. *Korean Journal of Parasitology*, *47*(SUPPL.), 103–114. https://doi.org/10.3347/kjp.2009.47.S.S103

Sohn WM, Yong TS, Eom KS, Pyo KH, Lee MY, Lim H, Choe S, Jeong HG, Sinuon M, Socheat D, C. J. (2012). Prevalence of Opisthorchis viverrini infection in humans and fish in Kratie Province, Cambodia. *Acta tropica*. 124(3), 215-20.

Sohn WM. (2009). Fish-borne zoonotic trematode metacercariae in the Republic of Korea. *The Korean journal of parasitology*, 47(Suppl), S103.

Sripa B, Bethony JM, Sithithaworn P, Kaewkes S, Mairiang E, Loukas A, Mulvenna J, Laha T, Hotez PJ, B. P. (2011). Opisthorchiasis and Opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta tropica*, 1(120), S158-68.

Sripa B, Kaewkes S, Intapan PM, Maleewong W, B. P. (2010). Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. *In Advances in parasitology*, 72, 305-350. *Academic Press.*

Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, Pairojkul C, Bhudhisawasdi V, Tesana S, Thinkamrop B, B. J. (2007). Liver fluke induces cholangiocarcinoma. *PLoS Med.* 4(7),e201.

Sripa B, P. C. (2008). Cholangiocarcinoma: lessons from Thailand. *Current opinion in gastroenterology*, 24(3), 349.

Srisawangwong T, Sithithaworn P, T. S. (1997). Metacercariae isolated from cyprinoid fishes in Khon Kaen District by digestion technic. *Southeast Asian journal of tropical medicine and public health*, 28, 224-6.

Sukontason, K. L., Sukontason, K., Boonsriwong, N., Chaithong, U., & Piangjai, S. (2001). Intensity of trematode metacercariae in cyprinoid fish in Chiang Mal Province, Northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *32*(SUPPL. 2), 214–217.

Sukontason KL, Sukontason K, Boonsriwong N, Chaithong U, P. S. (2001). Intensity of trematode metacercariae in cyprinoid fish in Chiang Mal Province, northern Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 32, 214-7.

Suwannatrai A, Saichua P, H. M. (2018). Epidemiology of Opisthorchis viverrini infection. *In Advances in Parasitology*, 101, 41-67. *Academic Press.*

Touch S, Yoonuan T, Nuamtanong S, Homsuwan N, Phuphisut O, Thaenkham U, W. J. (2013). Seasonal variation of Opisthorchis viverrini metacercarial infection in cyprinid fish from Southern Cambodia. *The Journal of Tropical Medicine and Parasitology, 36(1), 1-7.*

Touch, S., Komalamisra, C., Radomyos, P., & Waikagul, J. (2009). Discovery of Opisthorchis viverrini metacercariae in freshwater fish in southern Cambodia. *Acta Tropica*, 111(2), 108–113. https://doi.org/10.1016/j.actatropica.2009.03.002

Van Van K, Dalsgaard A, Blair D, L. T. (2009). Haplorchis pumilio and H. taichui in Vietnam discriminated using ITS-2 DNA sequence data from adults and larvae. *Experimental parasitology*, 123(2), 146-51.

Wattanayingcharoenchai S, Nithikathkul C, Wongsaroj T, Royal L, R. P. (2011). Geographic information system of Opisthorchis viverrini in northeast Thailand. *Asian Biomedicine*, 5(5), 687-91.

Wongratanacheewin S, Sermswan RW, S. S. (2003). Immunology and molecular biology of Opisthorchis viverrini infection. *Acta tropica*, 88(3), 195-207.

Wongsawad C, Phalee A, Noikong W, Chuboon S, N. C. (2012). Co-infection with Opisthorchis viverrini and Haplorchis taichui detected by human fecal examination in Chomtong district, Chiang Mai Province, Thailand. *Parasitology international, 61(1), 56-9.*

Wongsawad C, Rojanapaibul A, Mhad-arehin N, Pachanawan A, Marayong T, Suwattanacoupt S, Rojtinnakorn J, Wongsawad P, Kumchoo K, N. A. (2000). Metacercaria from freshwater fishes of Mae Sa stream, Chiang Mai, Thailand. *The Southeast Asian journal of tropical medicine and public health*, 31, 54.

Yamagishi J, Wakaguri H, Sugano S, Kawano S, Fujisaki K, Sugimoto C, Watanabe J, Suzuki Y, Kimata I, X. X. (2011). Construction and analysis of full-length cDNA library of Cryptosporidium parvum. *Parasitology international*, 60(2), 199-202.

Yong TS, Chai JY, Sohn WM, Eom KS, Jeoung HG, Hoang EH, Yoon CH, Jung BK, Lee SH, Sinuon M, S. D. (2014). Prevalence of intestinal helminths among inhabitants of Cambodia (2006-2011). *The Korean journal of parasitology*, 52(6), 661.

Young, N. D., Nagarajan, N., Lin, S. J., Korhonen, P. K., Jex, A. R., Hall, R. S., … Gasser, R. B. (2014). The Opisthorchis viverrini genome provides insights into life in the bile duct. *Nature Communications*, *5*, 1–11. https://doi.org/10.1038/ncomms5378

Zheng S, Zhu Y, Zhao Z, Wu Z, Okanurak K, L. Z. (2017). *Liver fluke infection and cholangiocarcinoma: a review. Parasitology research*. 116(1), 11-9.

APPENDIX

1.*Barbonymus gonionotus* (Silver barb)

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243); Tropical 22°C - 28°C

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Java (Ref. 27732). Occurs throughout the whole stretch on the Mekong, from the delta around the saline intrusion zone to Chiang Khong in Thailand (Ref. 37770).

Size

Max length: 40.5 cm TL male/unsexed; (Ref. 8609)

Description

Dorsal Spine (total): 4; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 6 - 7. Body is strongly compressed. The back is elevated, its dorsal profile arched, often concave above the occiput. The head is small; the snout pointed; the mouth terminal. The barbels are very minute or rudimentary, especially the upper ones, which sometimes disappear entirely. Color when fresh is silvery white, sometimes with a golden tint. The dorsal and caudal fins are gray to gray-yellow; the anal and pelvic fins light orange, their tips reddish; the pectoral fins pale to light yellow (Ref. 4792). Very few tubercles on the snout which are not visible without

magnification; snout length much less than the width of the eye socket (Ref. 37768). Anal-fin with 6-7 branches rays (Ref. 12693).

Biology

Occurs at midwater to bottom depths in rivers, streams, floodplains, and occasionally in reservoirs. Seems to prefer standing water habitats instead of flowing waters. Inhabits the flooded forest during high water period (Ref. 12693). Feeds on plant matter (e.g. leaves, weeds, Ipomea reptans and Hydrilla) and invertebrates (Ref. 4835). A migratory species but not considered to be a long-distance migrant. Regarded as local migrant which moves from the Mekong up into small streams and canals and onto flooded areas during the rainy season and back again during receding water (Ref. 37770). Some reports indicated that upstream migration of this fish is triggered by the first rains and rising water levels. When it finds a tributary, canal or stream it moves upstream and eventually onto flooded areas. When water recedes, it migrates back into canals and streams and into the Mekong again (Ref. 37770). Often used as a pituitary donor for artificial propagation in aquaculture. Escapees from culture installations have become established in rivers and form the basis for capture fisheries on several Southeast Asian islands (Ref. 1739). Useful in cropping excessive vegetation in reservoirs (Ref. 2686). Used for lap pa (in the preparation of which the numerous small bones are ground fine) or grilled or used to make som pa. Usually marketed fresh and occasionally seen in the aquarium trade (Ref. 12693). A specimen measuring 45 cm TL (2,100 g) was reportedly caught from Dan Tchang Reservoir, Thailand on 8 July 2003 (Jean-Francois Helias, pers. comm., FISHING ADVENTURES THAILAND [mailto:fishasia@ksc.th.com]).

2.*Puntioplites falcifer*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Puntioplites: Latin, punctum = point, the point of the sword + Greek, hoplon = weapon (Ref. 45335).

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong basin

Size

Max length: 40.0 cm male/unsexed; (Ref. 37770)

Distribution

Last simple anal ray osseus and serrated posteriorly; pale orange anal, pelvic and dorsal fins; last simple dorsal ray reaching the caudal fin in adults and with 28-36 serrae (in specimens 6-15 cm SL) (Ref. 27732); serrated anal spine; silvery body coloration (Ref. 12693).

Biology

Inhabits large upland rivers. Seems to avoid standing water. Little is known about its biology. Like other members of the genus, it probably feeds mainly on plant matter and occasionally on insects and insect larvae (Ref. 12693). Also reported to prefer deep pools in the river and to migrate into streams, canals and lakes during the flood season. Migrates in large schools (Ref. 37770). Migrates together with

Cosmochilus harmandi, Cirrhinus spp., Labeo chrysophekadion and Bangana sp (Ref. 37770). Marketed fresh (Ref. 12693).

3. *Mystacoleucus marginatus*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Mystacoleucus: Greek, mystax = whiskered, used by Belon in 1553 to describe all fishes with whiskers + Greek, leykos = white (Ref. 45335).

Environment

Freshwater; benthopelagic. Tropical; 22°C - 27°C (Ref. 2059)

Distribution

Asia: Mekong, Chao Phraya and Meklong basins; also from the Malay Peninsula, Borneo, Sumatra and Java. Recorded from Myanmar (Ref. 4832)

Size

Max length: 20.0 cm (Ref. 7050); common length: 10.0 cm (Ref. 12693)

Description

Anal fin with a convex distal margin; dorsal fin with black anterior and distal margins; a black distal margin on the caudal fin; most scales with a black crescent-shaped mark on their base (Ref. 27732).

Biology

Found at bottom depths of rivers and streams. Inhabits areas with sand or pea-gravel from small streams to large rivers (Ref. 12693). Usually found in streams with clear and moving waters (Ref. 27732). Breeds when water levels begin to rise, but whether it leaves permanent water or not is unknown. Occasionally seen in markets (Ref. 12693).

4. *Anematichthys repasson*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java and Borneo.

Size

Max length: 28.0 cm (Ref. 7050)

Description

Has a black blotch at the base of the caudal fin, which is not always distinct; rows of spots along scale rows; two pairs of barbels (Ref. 27732).

Biology

Found in midwater to bottom levels in small rivers, canals, ponds and reservoirs (Ref. 12693). Occurs in medium to large-sized rivers (Ref. 12975). Moves out into flooded forest during high-water season. (Ref. 12693).

5. *Systomus Rubripinnis*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Tropical Freshwater; benthopelagic; Tropical; 22°C - 25°C (Ref. 1672)

Distribution

Asia: Chao Phraya, Mekong and Mae Khlong basins, Malay Peninsula and Indonesia

Size

Max length: 25.0 cm (Ref. 30857)

Description

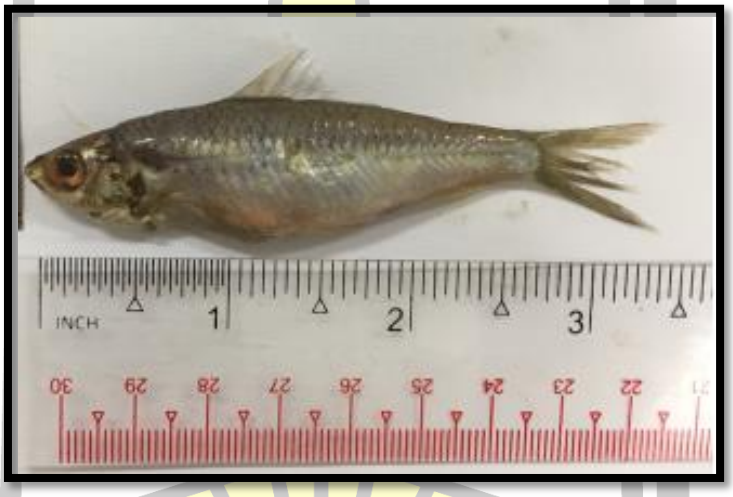
Dorsal spines (total): 4; Dorsal soft rays (total): 8; Anal spines: 2; Anal soft rays: 5. With 4 barbels (Ref. 43281); anterior maxillary ("rostral") barbels present (Ref. 12693); last simple dorsal ray osseous and serrated posteriorly; conspicuous black blotch on caudal peduncle; small, vertically elongated, black spot below dorsal origin; narrow dark band from upper extremity of gill opening to pectoral base; caudal

fin orange red with a conspicuous broad black marginal stripe on each lobe; usually longitudinal rows of black spots along scale rows (Ref. 43281)

Biology

Occurs in rivers of all sizes, but mainly in smaller streams, canals and on floodplains. Occasionally found in impoundments, but usually stays in the flowing streams leading to the impoundment. Moves into seasonally inundated areas and breeds at the start of the rainy season. Young of the year appear in streams in July and August. Adults leave the floodplains when the water recedes in December or January (Ref. 12693). Captured from the wild for the ornamental fish trade in Thailand (Ref. 6459). Marketed fresh or made into prahoc along the Tonle Sap (Ref. 12693).

6.*Cyclocheilichthys repasson*



Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java and Borneo.

Size

Max length: 28.0 cm (Ref. 7050)

Description

Has a black blotch at the base of the caudal fin, which is not always distinct; rows of spots along scale rows; two pairs of barbels (Ref. 27732).

Biology

Found in midwater to bottom levels in small rivers, canals, ponds and reservoirs (Ref. 12693). Occurs in medium to large-sized rivers (Ref. 12975). Moves out into flooded forest during high-water season. (Ref. 12693).

7. *Anabas testudineus*

Classification

Anabantidae (Climbing gouramies)

Etymology: Anabas: Greek, anabasis = climbing up (Ref. 45335).

Environment

Freshwater; brackish; demersal; potamodromous (Ref. 51243). Tropical; 22°C - 30°C (Ref. 1672)

Distribution

Asia: India to Wallace line including China. May have been distributed in more areas than were commonly reported.

Size

Max length: 25.0 cm (Ref. 4833); common length: 12.5 cm (Ref. 2686)

Description

Dorsal spines (total): 16 - 20; Dorsal soft rays (total): 7-10; Anal spines: 9-11; Anal soft rays: 8 - 11. Color in life dark to pale greenish, very pale below, back dusky to olive; head with longitudinal stripes ventrally; posterior margin of opercle with a dark spot; iris golden reddish. Body form variable, affected by age and amount of food consumed. Scaled head with 4-5 rows between eye and rear margin of preoperculum. Scales large and regularly arranged, ciliate.

Biology

Found mostly in canals, lakes, ponds, swamps and estuaries (Ref. 41236, 57235). Adults occur in medium to large rivers, brooks, flooded fields and stagnant water bodies including sluggish flowing canals (Ref. 12975). Often found in areas with dense vegetation (Ref. 12693). Can tolerate extremely unfavorable water conditions and is associated mainly with turbid, stagnant waters (Ref. 6028). They remain buried under the mud during dry season (Ref. 1479). Feed on macrophytic vegetation, shrimps and fish fry (Ref. 6028). Reported to undertake lateral migration from the Mekong mainstream, or other permanent water bodies, to flooded areas during the flood season and return to the permanent water bodies at the onset of the dry season (Ref. 37770). During the dry season, they stay in pools associated with submerged woods and shrubs (Ref. 37770). Posses an accessory air-breathing organ (Ref. 2847). Able to survive for several days or weeks out of water if the air breathing organs can be kept moist (Ref. 1479). Quite famous for its ability to walk; important food fish in SE Asia, considered as a tasty food fish (Ref. 6565) but not of the finest quality since it is bony (Ref. 2686). Usually sold live in markets where it is kept alive for several days by keeping it moist (Ref. 12693). Economic foodfish in the Southeast Asia (Ref. 57235).

8.*labiobarbus siamensis*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Labiobarbus: Latin, labium = lip + Latin, barbus = barbel (Ref. 45335).

Environment

Freshwater; benthopelagic. Tropical

Distribution

Asia: Chao Phraya, Bankpakong and Mekong basins.

Size

Max length: 22.0 cm TL male/unsexed; (Ref. 12693)

Description

Dorsal soft rays (total): 30. Presence of dark mark above pectoral fin forming a well-defined ring (often absent); 25-30 branched dorsal rays; caudal and dorsal dusky, without well-defined color pattern (Ref. 43281)

Biology

Occur in midwater to bottom levels of rivers and streams. Migrates out into flooded forests during high water periods where it feeds on phytoplankton, periphyton, benthic algae and some zooplankton. Used to make prahoc (Ref. 12693)

9.*Henicorhynchus siamensis*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong and Chao Phraya basins.

Size

Max length: 20.0 cm (Ref. 33488)

Description

Vertebrae: 33 - 34. Head large and broad; relatively deep body, snout not or weakly projecting; plain silvery body (Ref. 43281)

Biology

Often found in great abundance at midwater to bottoms depths in large and small rivers. Feeds on algae, periphyton and phytoplankton. Not known to prosper in impoundments. Well known for its annual trophic migrations out to the floodplains in wet season. Returns to rivers as water levels begin to fall in October with numbers increasing through December and then slowly declining (Ref. 12693). From just upstream Phnom Penh in Cambodia to the Khone Falls this species is reported to migrate upstream during the period October-February. At Muk Kompul in Kandal Province, it migrates upstream just before the full moon. Further upstream near Kratie, migration occurs during full moon and at Sambor, migration takes place

immediately after full moon. Near the Khone Falls, upstream movements continue through March but in April fish are moving in both directions. From May to July, at the start of the rainy season, it migrates downstream from the Khone Falls to the Mekong Delta. Here, the fish is reported to move out of the Mekong into canals and flooded areas in August-September. When water recedes in November-December, fish migrates to the Mekong again. Upstream the Khone Falls near Ubolratchatani in Thailand, this species moves upstream between February and June, consisting mainly of juveniles in February-March and of adults (15-20 cm) in April-June. Further upstream from Xayabouri in Laos to Chiang Khong in Thailand, upstream migrations take place between March to July, first by juveniles, later by adults (Ref. 37770). Used to make prahoc along the Tonlé Sap, Cambodia. Often seen in the aquarium trade (Ref. 12693).

10. *Mystacoleucus marginatus*

Classification

Order: Cyprini formes

Family: Cyprinidae

Etymology: Mystacoleucus: (Ref. 45335).

Environment

Freshwater; benthopelagic. Tropical; 22°C - 27°C (Ref. 2059)

Distribution

Asia: Mekong, Chao Phraya and Meklong basins; also from the Malay Peninsula, Borneo, Sumatra and Java. Recorded from Myanmar (Ref. 4832).

Size

Max length: 20.0 cm (Ref. 7050); common length: 10.0 cm SL (Ref. 12693)

Description

Anal fin with a convex distal margin; dorsal fin with black anterior and distal margins; a black distal margin on the caudal fin; most scales with a black crescent-shaped mark on their base (Ref. 27732)

Biology

Found at bottom depths of rivers and streams. Inhabits areas with sand or pea-gravel from small streams to large rivers (Ref. 12693). Usually found in streams with clear and moving waters (Ref. 27732). Breeds when water levels begin to rise, but whether it leaves permanent water or not is unknown. Occasionally seen in markets (Ref. 12693)

11. *Rasbora argyrotaenia*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Rasbora: Rasbora, an Indian word for a fish, also used in Malay peninsula.

Environment

Freshwater; benthopelagic. Tropical; 20°C - 26°C (Ref. 2060)

Distribution

Asia: Mekong, Chao Phraya and Mae Khlong basins, Malay Peninsula to Borneo, Java and Sumatra in Indonesia.

Size

Max length: 12.0 cm (Ref. 6398)

Description

Dorsal spines (total): 2; Dorsal soft rays (total): 7; Anal spines: 3; Anal soft rays: 5. Preserved color yellowish brown with silvery sheen, darker dorsally; scales margined by brown lines or dots. 12-13 scales between nape and dorsal. Lateral line complete reaching caudal; 9 (rarely 8) scale rows between lateral lines over middle of caudal peduncle. Origin of dorsal between tip of snout and caudal; least depth of caudal peduncle 1.9 - 2 in its length. Maxillary not reaching eye. Thin lips, upper moderately protractile; lower sometimes with external projecting knob at symphysis, fitting the notch on upper one.

Biology

Occurs mainly in rivers and enters flooded fields (Ref. 12975). Feeds on algae (Ref. 12975).

12.*Cyclocheilichthys repasson*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java and Borneo.

Size

Max length: 28.0 cm (Ref. 7050)

Description

Has a black blotch at the base of the caudal fin, which is not always distinct; rows of spots along scale rows; two pairs of barbels (Ref. 27732).

Biology

Found in midwater to bottom levels in small rivers, canals, ponds and reservoirs (Ref. 12693). Occurs in medium to large-sized rivers (Ref. 12975). Moves out into flooded forest during high-water season. (Ref. 12693).

13.*Systomus orphoides*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; Tropical

Distribution

Asia: locality is ‘Java, Indonesia’, but this species is currently considered to have a wide range extending eastwards from northern India and Myanmar via Thailand, Laos, and Cambodia as far as Vietnam and to the south through Peninsular Malaysia and the Greater Sunda Islands of Borneo, Sumatra, and Java. https://www.seriouslyfish.com/species/systomus-orphoides/It thus occurs throughout the great Mae Klong, Chao Phraya and Mekong drainages as well as many smaller river basins, lakes and reservoirs. More recently it’s recorded close to Chennai in Tamil Nadu state, southern India but it remains to be seen whether it’s more widely-distributed than previously thought or has been introduced in some way (Knight and Rema devi 2010).

Size

Max length: 200 – 250 mm.

https://www.seriouslyfish.com/species/systomus-orphoides/

Description

Reference photo in https://www.seriouslyfish.com/species/systomus-orphoides/

Biology

Mostly inhabits flowing tributaries, large streams, and their surrounding floodplains. he fish move into inundated agricultural areas and forests during the wet season to feed and spawn, returning to the rivers as the water begins to recede. It's also found in some larger rivers and permanent water bodies including the highly seasonal Tonlé Sap system in Cambodia.

14.*Puntius brevis*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Puntius: Lantin, punctum = point, the point of the sword (Ref. 45335)

Environment

Freshwater; benthopelagic; Tropical

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula and Java.

Size

Max length: 12.0 cm SL male/unsexed; (Ref. 30857)

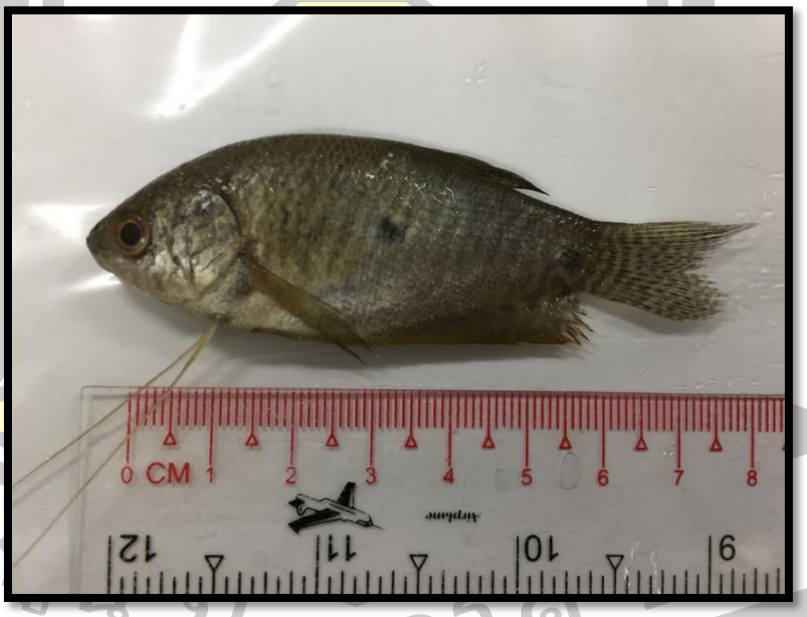
Description

The last simple dorsal ray without serration along its posterior margin; 1 pair of maxillary barbels; a black blotch on caudal peduncle (Ref. 27732); lateral line complete; no discrete dark spot at base of anterior dorsal-fin rays (Ref. 12693)

Biology

Usually found in slow moving or standing water (Ref. 27732). Occurs in floodplains, canals, ditches and small sluggish streams. Proliferates in impoundments, and lives in areas with abundant aquatic vegetation. Feeds on crustaceans, tubificid worms, algae and zooplankton. Moves into newly inundated land during flood season and spawns there (Ref. 12693, 57235).

15.*Trichogaster trichopterus*



Classification

Family: Osphronemidae

Environment

Freshwater; benthopelagic; pH range: 6.0 - 8.0; dH range: 5 - 19; potamodromous (Ref. 51243). Tropical; 22°C - 28°C (Ref. 1672)

Distribution

Asia: Mekong basin in Laos, Yunnan, Thailand, Cambodia and Viet Nam; Southeast Asia (Ref. 27732).

Size

Max length: 15.0 cm (Ref. 30857); common length: 10.0 cm (Ref. 57235)

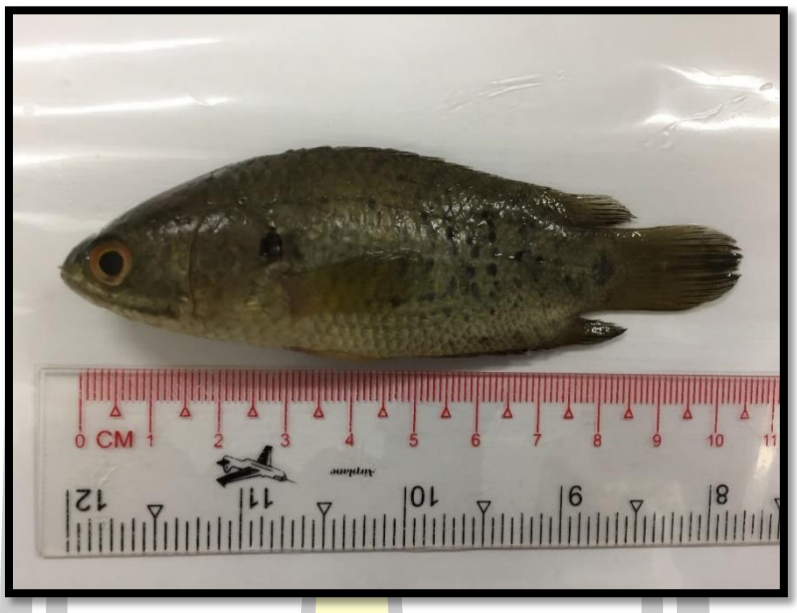
Description

Dorsal spines (total): 6 - 8; Dorsal soft rays (total): 7-10; Anal spines: 9-12; Anal soft rays: 30 - 38. Color in life brown; shoulders with irregular dark marks, yellowish on opercles and thorax; median fins and pectorals brown, ventrals yellowish. Mouth very small, very oblique, upper jaw vertical and somewhat protractile, lower jaw prominent. Scales of moderate size, irregularly arranged. Lateral line curved, irregular. Caudal fin slightly emarginate or truncate (Ref. 4792). With 8-9 dorsal-fin branched rays; 33-38 branched anal-fin rays; black spot in middle of side and at caudal-fin base (Ref. 12693). Body with numerous narrow irregular oblique bars (Ref. 43281)

Biology

Lives in lowland wetlands (Ref. 57235). Found in marshes, swamps and canals (Ref. 43281). Inhabits shallow sluggish or standing-water with a lot of aquatic vegetation. Occurs in seasonally flooded forests throughout the middle and lower Mekong (Ref. 12693). Undertakes lateral migrations from the Mekong mainstream, or other permanent water bodies, to flooded areas during the flood season and returns to the permanent water bodies at the onset of the dry season (Ref. 37770). An air-breathing species (Ref. 118402). Feeds on zooplankton, crustaceans and insect larvae. Processed into salted, dried fish in Java (Ref. 4929). Also marketed fresh and commonly seen in the aquarium fish trade (Ref. 12693). Aquarium keeping: minimum aquarium size 100 cm (Ref. 51539).

16. *Anabas testudineus*



Classification

Anabantidae (Climbing gouramies)

Etymology: Anabas: Greek, anabasis = climbing up (Ref. 45335).

Environment

Freshwater; brackish; demersal; potamodromous (Ref. 51243). Tropical; 22°C - 30°C (Ref. 1672)

Distribution

Asia: India to Wallace line including China. May have been distributed in more areas than were commonly reported.

Size

Max length: 25.0 cm (Ref. 4833); common length: 12.5 cm (Ref. 2686)

Description

Dorsal spines (total): 16 - 20; Dorsal soft rays (total): 7-10; Anal spines: 9-11; Anal soft rays: 8 - 11. Color in life dark to pale greenish, very pale below, back dusky to olive; head with longitudinal stripes ventrally; posterior margin of opercle with a dark spot; iris golden reddish. Body form variable, affected by age and amount of food consumed. Scaled head with 4-5 rows between eye and rear margin of preoperculum. Scales large and regularly arranged, ciliate.

Biology

Found mostly in canals, lakes, ponds, swamps and estuaries (Ref. 41236, 57235). Adults occur in medium to large rivers, brooks, flooded fields and stagnant water bodies including sluggish flowing canals (Ref. 12975). Often found in areas with dense vegetation (Ref. 12693). Can tolerate extremely unfavorable water conditions and is associated mainly with turbid, stagnant waters (Ref. 6028). They remain buried under the mud during dry season (Ref. 1479). Feed on macrophytic vegetation, shrimps and fish fry (Ref. 6028). Reported to undertake lateral migration from the Mekong mainstream, or other permanent water bodies, to flooded areas during the flood season and return to the permanent water bodies at the onset of the dry season (Ref. 37770). During the dry season, they stay in pools associated with submerged woods and shrubs (Ref. 37770). Posses an accessory air-breathing organ (Ref. 2847). Able to survive for several days or weeks out of water if the air breathing organs can be kept moist (Ref. 1479). Quite famous for its ability to walk; important food fish in SE Asia, considered as a tasty food fish (Ref. 6565) but not of the finest quality since it is bony (Ref. 2686). Usually sold live in markets where it is kept alive for several days by keeping it moist (Ref. 12693). Economic foodfish in the Southeast Asia (Ref. 57235).

17.*Balantiocheilos melanopterus*

Classification

Family: Cyprinidae

Etymology: Balantiocheilos: Greek, balantion = bag + Greek, cheilos = lip (Ref. 45335). More on author: Bleeker.

Environment

Freshwater; benthopelagic; pH range: 6.0 - 8.0; dH range: 5 - 12. Tropical; 22°C - 28°C (Ref. 1672)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Borneo. Becoming rare or extinct in many river basins.

Size

Max length: 35.0 cm SL male/unsexed; (Ref. 30857)

Description

The Silver body with black margins on dorsal, caudal, anal and pelvic; lower lip with a posterior groove forming a pocket opening backwards (Ref. 43281).

Biology

Found in midwater depths in large and medium-sized rivers and lakes. Feeds on phytoplankton, but mostly on small crustaceans, rotifers as well as insects and their larvae (Ref. 12693). Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size >150 cm (Ref. 51539).

18. *Barbonymus schwanenfeldii*

Classification

Family: Cyprinidae

Environment

Freshwater; benthopelagic; pH range: 6.5 - 7.0; potamodromous (Ref. 51243). Tropical; 22°C - 25°C (Ref. 13371)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Borneo.

Size

Max length: 35.0 cm (Ref. 30857); common length: 20.0 cm (Ref. 4835)

Description

Dorsal spines (total): 3; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 5. Distinguished from other species of the genus in having a red dorsal fin with a black blotch at the tip, red pectoral, pelvic and anal fins, red caudal fin with white margin and a black submarginal stripe along each lobe, and 8 scale rows between dorsal-fin origin and lateral line (Ref. 27732). Large individuals silvery or golden yellow in life with its dorsal fin red and caudal fin orange or blood-red (Ref. 2091)

Biology

Found in rivers, streams, canals and ditches (Ref. 12693). Occurs in medium to large-sized rivers and enters flooded fields (Ref. 12975). In east Kalimantan, Indonesia a temperature range of 20.4-33.7°C was recorded for this species (Ref. 6129). Largely herbivorous, consuming aquatic macrophytes and submerged land

plants, as well as filamentous algae and occasionally insects (Ref. 12693). Also feeds on small fishes (Ref. 12693), worms and crustaceans (Ref. 7020). Usually marketed fresh (Ref. 12693).

19. *Systomus orphoides*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; Tropical

Distribution

Asia: locality is ‘Java, Indonesia’, but this species is currently considered to have a wide range extending eastwards from northern India and Myanmar via Thailand, Laos, and Cambodia as far as Vietnam and to the south through Peninsular Malaysia and the Greater Sunda Islands of Borneo, Sumatra, and Java. https://www.seriouslyfish.com/species/systomus-orphoides/It thus occurs throughout the great Mae Klong, Chao Phraya and Mekong drainages as well as many smaller river basins, lakes and reservoirs. More recently it’s recorded close to Chennai in Tamil Nadu state, southern India but it remains to be seen whether it’s more widely-

distributed than previously thought or has been introduced in some way (Knight and Rema devi 2010).

Size

Max length: 200 – 250 mm.

https://www.seriouslyfish.com/species/systomus-orphoides/

Description

Reference photo in https://www.seriouslyfish.com/species/systomus-orphoides/

Biology

Mostly inhabits flowing tributaries, large streams, and their surrounding floodplains. he fish move into inundated agricultural areas and forests during the wet season to feed and spawn, returning to the rivers as the water begins to recede. It's also found in some larger rivers and permanent water bodies including the highly seasonal Tonlé Sap system in Cambodia.

20. *Balantiocheilos ambusticauda*

Classification

Family: Cyprinidae

Etymology: *Balantiocheilos*: Greek, balantion = bag + Greek, cheilos = lip (Ref. 45335); ambusticauda: Name from the Latin ambustus, meaning burned around, scorched and cauda for tail; referring to the black edge of the caudal fin

Environment

Tropical

Distribution

Southeast Asia: Lower and middle Mekong and Chao Phraya river drainages in mainland Southeast Asia. The original distribution of this species included the Chao Phraya River drainage from Bangkok upriver to the lower Nan River (Smith, 1945) and in the Mekong from Viet Nam and the Great Lake (Tonle Sap) to the lower Nam Ngum River (Taki, 1968). The specimen identified as Balantiocheilus from Chiengmai by Fowler (1934: 127) is actually a juvenile Poropuntius

Size

Max length: 10.5 cm (Ref. 58339)

Description

Balantiocheilos ambusticauda is distinguished from its sole congener, *B. melanopterus*, in having a shorter snout that is rounded (vs. obliquely truncate), posteriorly directed groove at rictus curved (vs. straight), and narrower black margins on the pelvic and anal fins (on distal third of both fins or less vs. on distal half or more, with pelvic fins sometimes entirely black) (Ref. 58339).

21. *Hampala Dispar*

Classification

Family: Cyprinidae

Etymology: Hampala: Local name from Java

Environment

Tropical

Distribution

Asia: endemic to the Mekong basin.

Size

Max length :35.0 cm (Ref. 30857)

Description

Has a round midlateral blotch under the dorsal-fin origin in adults and a plain grey caudal fin (Ref. 27732); juveniles lack black teardrop-shaped marking on cheek; barbel always shorter than eye width (Ref. 12693).

Biology

Occurs in slowly moving or standing water habitats (Ref. 12693). Encountered also in rapid-running mountain streams of the middle Mekong (Ref. 12975). Common in impoundments, with small individuals frequenting areas of dense vegetation. Feeds mainly on prawns, crabs, and shrimps, along with some insect larvae and some fish as well. Breeds at the start of the rainy season and the young are found in seasonally flooded habitats in June. Marketed fresh (Ref. 12693).

22. *Hampala macrolepidota*

Classification

Family: Cyprinidae

Etymology: Hampala: Local name from Java

Environment

Tropical; 22°C - 25°C (Ref. 2059)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula and Indonesia

Size

Max length -70.0 cm (Ref. 2686); common length: 35.0 cm (Ref. 12693)

Description

Has a black bar between the dorsal and pelvic fins in adults; orange to red caudal fin with a black longitudinal, marginal stripe along each lobe; juveniles usually with an additional vertical bar on the caudal peduncle (Ref. 27732); juveniles with black teardrop-shaped marking on cheek; barbel always longer than eye width (Ref. 12693). Eyes located in upper-side of head; with a vertical groove reaching behind mouth in isthmus; branchial membranes connected and free (Ref. 45536).

Biology

Occurs mainly in clear rivers or streams with running water and sandy to muddy bottoms (Ref. 4832). Found in most water bodies, except small creeks, torrents, and shallow swamps (Ref. 27732, 57235). A migratory species (Ref. 37772). Enters flooded forest (Ref. 9497). Abundant in the Mekong and in the Nam Ngum Reservoir. Found in the basin-wide tributary of the lower Mekong (Ref. 36667). Largest individual believed to be in a pool below the Chendoroh Dam in Perah, Malaysia. A predatory fish: adults mainly piscivorous in Zoo Negara Lake, Malaysia, but in Saguling Reservoir, West Java, 74 % of the diet consists of aquatic insects. Breeds throughout the rainy season (Ref. 12693). Good fish though bony. May be made into `Lap pa'. Marketed fresh (Ref. 12693). Commonly seen in markets; economic important foodfish (Ref. 57235).

23.*Barbonymus gonionotus*

Classification

Family: Cyprinidae

Environment

Freshwater; benthopelagic; pH range: 6.5 - 7.0; potamodromous (Ref. 51243). Tropical; 22°C - 25°C (Ref. 13371)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Borneo.

Size

Max length: 35.0 cm (Ref. 30857); common length: 20.0 cm (Ref. 4835)

Description

Dorsal spines (total): 3; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 5. Distinguished from other species of the genus in having a red dorsal fin with a black blotch at the tip, red pectoral, pelvic and anal fins, red caudal fin with white margin and a black submarginal stripe along each lobe, and 8 scale rows between dorsal-fin origin and lateral line (Ref. 27732). Large individuals silvery or golden yellow in life with its dorsal fin red and caudal fin orange or blood-red (Ref. 2091)

Biology

Found in rivers, streams, canals and ditches (Ref. 12693). Occurs in medium to large-sized rivers and enters flooded fields (Ref. 12975). In east Kalimantan, Indonesia a temperature range of 20.4-33.7°C was recorded for this species (Ref. 6129). Largely herbivorous, consuming aquatic macrophytes and submerged land plants, as well as filamentous algae and occasionally insects (Ref. 12693). Also feeds on small fishes (Ref. 12693), worms and crustaceans (Ref. 7020). Usually marketed fresh (Ref. 12693).

24. *Puntioplites falcifer*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Puntioplites: Latin, punctum = point, the point of the sword + Greek, hoplon = weapon (Ref. 45335).

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong basin

Size

Max length: 40.0 cm male/unsexed; (Ref. 37770)

Distribution

Last simple anal ray osseus and serrated posteriorly; pale orange anal, pelvic and dorsal fins; last simple dorsal ray reaching the caudal fin in adults and with 28-36 serrae (in specimens 6-15 cm SL) (Ref. 27732); serrated anal spine; silvery body coloration (Ref. 12693).

Biology

Inhabits large upland rivers. Seems to avoid standing water. Little is known about its biology. Like other members of the genus, it probably feeds mainly on plant matter and occasionally on insects and insect larvae (Ref. 12693). Also reported to

prefer deep pools in the river and to migrate into streams, canals and lakes during the flood season. Migrates in large schools (Ref. 37770). Migrates together with Cosmochilus harmandi, Cirrhinus spp., Labeo chrysophekadion and Bangana sp (Ref. 37770). Marketed fresh (Ref. 12693).

25. *Barbonymus gonionotus*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243); Tropical 22°C - 28°C

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Java (Ref. 27732). Occurs throughout the whole stretch on the Mekong, from the delta around the saline intrusion zone to Chiang Khong in Thailand (Ref. 37770).

Size

Max length: 40.5 cm TL male/unsexed; (Ref. 8609)

Description

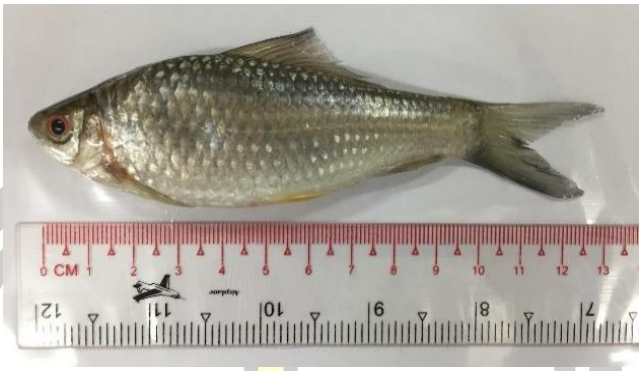
Dorsal Spine (total): 4; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 6 - 7. Body is strongly compressed. The back is elevated, its dorsal profile arched, often concave above the occiput. The head is small; the snout pointed; the mouth terminal. The barbels are very minute or rudimentary, especially the upper

ones, which sometimes disappear entirely. Color when fresh is silvery white, sometimes with a golden tint. The dorsal and caudal fins are gray to gray-yellow; the anal and pelvic fins light orange, their tips reddish; the pectoral fins pale to light yellow (Ref. 4792). Very few tubercles on the snout which are not visible without magnification; snout length much less than the width of the eye socket (Ref. 37768). Anal-fin with 6-7 branches rays (Ref. 12693).

Biology

Occurs at midwater to bottom depths in rivers, streams, floodplains, and occasionally in reservoirs. Seems to prefer standing water habitats instead of flowing waters. Inhabits the flooded forest during high water period (Ref. 12693). Feeds on plant matter (e.g. leaves, weeds, Ipomea reptans and Hydrilla) and invertebrates (Ref. 4835). A migratory species but not considered to be a long-distance migrant. Regarded as local migrant which moves from the Mekong up into small streams and canals and onto flooded areas during the rainy season and back again during receding water (Ref. 37770). Some reports indicated that upstream migration of this fish is triggered by the first rains and rising water levels. When it finds a tributary, canal or stream it moves upstream and eventually onto flooded areas. When water recedes, it migrates back into canals and streams and into the Mekong again (Ref. 37770). Often used as a pituitary donor for artificial propagation in aquaculture. Escapees from culture installations have become established in rivers and form the basis for capture fisheries on several Southeast Asian islands (Ref. 1739). Useful in cropping excessive vegetation in reservoirs (Ref. 2686). Used for lap pa (in the preparation of which the numerous small bones are ground fine) or grilled or used to make som pa. Usually marketed fresh and occasionally seen in the aquarium trade (Ref. 12693). A specimen measuring 45 cm TL (2,100 g) was reportedly caught from Dan Tchang Reservoir, Thailand on 8 July 2003 (Jean-Francois Helias, pers. comm., FISHING ADVENTURES THAILAND [mailto:fishasia@ksc.th.com]).

26. Systomus *rubripinnis*



Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Tropical Freshwater; benthopelagic; Tropical; 22°C - 25°C (Ref. 1672)

Distribution

Asia: Chao Phraya, Mekong and Mae Khlong basins, Malay Peninsula and Indonesia

Size

Max length: 25.0 cm (Ref. 30857)

Description

Dorsal spines (total): 4; Dorsal soft rays (total): 8; Anal spines: 2; Anal soft rays: 5. With 4 barbels (Ref. 43281); anterior maxillary ("rostral") barbels present (Ref. 12693); last simple dorsal ray osseous and serrated posteriorly; conspicuous black blotch on caudal peduncle; small, vertically elongated, black spot below dorsal origin; narrow dark band from upper extremity of gill opening to pectoral base; caudal fin orange red with a conspicuous broad black marginal stripe on each lobe; usually longitudinal rows of black spots along scale rows (Ref. 43281)

Biology

Occurs in rivers of all sizes, but mainly in smaller streams, canals and on floodplains. Occasionally found in impoundments, but usually stays in the flowing streams leading to the impoundment. Moves into seasonally inundated areas and breeds at the start of the rainy season. Young of the year appear in streams in July and August. Adults leave the floodplains when the water recedes in December or January

(Ref. 12693). Captured from the wild for the ornamental fish trade in Thailand (Ref. 6459). Marketed fresh or made into prahoc along the Tonle Sap (Ref. 12693).

27. *Balantiocheilos ambusticauda*

Classification

Family: Cyprinidae

Etymology: *Balantiocheilos*: Greek, balantion = bag + Greek, cheilos = lip (Ref. 45335); ambusticauda: Name from the Latin ambustus, meaning burned around, scorched and cauda for tail; referring to the black edge of the caudal fin

Environment

Tropical

Distribution

Southeast Asia: Lower and middle Mekong and Chao Phraya river drainages in mainland Southeast Asia. The original distribution of this species included the Chao Phraya River drainage from Bangkok upriver to the lower Nan River (Smith, 1945) and in the Mekong from Viet Nam and the Great Lake (Tonle Sap) to the lower Nam Ngum River (Taki, 1968). The specimen identified as Balantiocheilus from Chiengmai by Fowler (1934: 127) is actually a juvenile Poropuntius

Size

Max length: 10.5 cm (Ref. 58339)

Description

Balantiocheilos ambusticauda is distinguished from its sole congener, *B. melanopterus*, in having a shorter snout that is rounded (vs. obliquely truncate),

posteriorly directed groove at rictus curved (vs. straight), and narrower black margins on the pelvic and anal fins (on distal third of both fins or less vs. on distal half or more, with pelvic fins sometimes entirely black) (Ref. 58339).

28. *Hampala dispar*

Classification

Family: Cyprinidae

Etymology: Hampala: Local name from Java

Environment

Tropical

Distribution

Asia: endemic to the Mekong basin.

Size

Max length :35.0 cm (Ref. 30857)

Description

Has a round midlateral blotch under the dorsal-fin origin in adults and a plain grey caudal fin (Ref. 27732); juveniles lack black teardrop-shaped marking on cheek; barbel always shorter than eye width (Ref. 12693).

Biology

Occurs in slowly moving or standing water habitats (Ref. 12693). Encountered also in rapid-running mountain streams of the middle Mekong (Ref. 12975). Common in impoundments, with small individuals frequenting areas of dense vegetation. Feeds mainly on prawns, crabs, and shrimps, along with some insect

larvae and some fish as well. Breeds at the start of the rainy season and the young are found in seasonally flooded habitats in June. Marketed fresh (Ref. 12693)

29. *Barbonymus schwanenfeldii*

Classification

Family: Cyprinidae

Environment

Freshwater; benthopelagic; pH range: 6.5 - 7.0; potamodromous (Ref. 51243). Tropical; 22°C - 25°C (Ref. 13371)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Borneo.

Size

Max length: 35.0 cm (Ref. 30857); common length: 20.0 cm (Ref. 4835)

Description

Dorsal spines (total): 3; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 5. Distinguished from other species of the genus in having a red dorsal fin with a black blotch at the tip, red pectoral, pelvic and anal fins, red caudal fin with white margin and a black submarginal stripe along each lobe, and 8 scale rows between dorsal-fin origin and lateral line (Ref. 27732). Large individuals silvery or golden yellow in life with its dorsal fin red and caudal fin orange or blood-red (Ref. 2091)

Biology

Found in rivers, streams, canals and ditches (Ref. 12693). Occurs in medium to large-sized rivers and enters flooded fields (Ref. 12975). In east Kalimantan, Indonesia a temperature range of 20.4-33.7°C was recorded for this species (Ref. 6129). Largely herbivorous, consuming aquatic macrophytes and submerged land plants, as well as filamentous algae and occasionally insects (Ref. 12693). Also feeds on small fishes (Ref. 12693), worms and crustaceans (Ref. 7020). Usually marketed fresh (Ref. 12693).

30. *Cyclocheilichthys repasson*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java and Borneo.

Size

Max length: 28.0 cm (Ref. 7050)

Description

Has a black blotch at the base of the caudal fin, which is not always distinct; rows of spots along scale rows; two pairs of barbels (Ref. 27732).

Biology

Found in midwater to bottom levels in small rivers, canals, ponds and reservoirs (Ref. 12693). Occurs in medium to large-sized rivers (Ref. 12975). Moves out into flooded forest during high-water season. (Ref. 12693).

31. *Barbonymus belinka*

Classification

Family: Cyprinidae

Environment

Freshwater; benthopelagic; Tropical

Distribution

Asia: Indonesia and Malaysia (Ref. 7050).

Size

23.5 cm (Ref. 7050)

Description

Kottelat, M. (2013): https://en.wikipedia.org/wiki/Barbonymus_belinka

32.*Hampala Dispar*

Classification

Family: Cyprinidae

Etymology: Hampala: Local name from Java

Environment

Tropical

Distribution

Asia: endemic to the Mekong basin.

Size

Max length :35.0 cm (Ref. 30857)

Description

Has a round midlateral blotch under the dorsal-fin origin in adults and a plain grey caudal fin (Ref. 27732); juveniles lack black teardrop-shaped marking on cheek; barbel always shorter than eye width (Ref. 12693).

Biology

Occurs in slowly moving or standing water habitats (Ref. 12693). Encountered also in rapid-running mountain streams of the middle Mekong (Ref. 12975). Common in impoundments, with small individuals frequenting areas of dense vegetation. Feeds mainly on prawns, crabs, and shrimps, along with some insect larvae and some fish as well. Breeds at the start of the rainy season and the young are found in seasonally flooded habitats in June. Marketed fresh (Ref. 12693).

33. *Barbonymus gonionotus*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243); Tropical 22°C - 28°C

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Java (Ref. 27732). Occurs throughout the whole stretch on the Mekong, from the delta around the saline intrusion zone to Chiang Khong in Thailand (Ref. 37770).

Size

Max length: 40.5 cm TL male/unsexed; (Ref. 8609)

Description

Dorsal Spine (total): 4; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 6 - 7. Body is strongly compressed. The back is elevated, its dorsal profile arched, often concave above the occiput. The head is small; the snout pointed; the mouth terminal. The barbels are very minute or rudimentary, especially the upper ones, which sometimes disappear entirely. Color when fresh is silvery white, sometimes with a golden tint. The dorsal and caudal fins are gray to gray-yellow; the anal and pelvic fins light orange, their tips reddish; the pectoral fins pale to light yellow (Ref. 4792). Very few tubercles on the snout which are not visible without magnification; snout length much less than the width of the eye socket (Ref. 37768). Anal-fin with 6-7 branches rays (Ref. 12693).

Biology

Occurs at midwater to bottom depths in rivers, streams, floodplains, and occasionally in reservoirs. Seems to prefer standing water habitats instead of flowing waters. Inhabits the flooded forest during high water period (Ref. 12693). Feeds on plant matter (e.g. leaves, weeds, Ipomea reptans and Hydrilla) and invertebrates (Ref. 4835). A migratory species but not considered to be a long-distance migrant. Regarded as local migrant which moves from the Mekong up into small streams and canals and onto flooded areas during the rainy season and back again during receding water (Ref. 37770). Some reports indicated that upstream migration of this fish is triggered by the first rains and rising water levels. When it finds a tributary, canal or stream it moves upstream and eventually onto flooded areas. When water recedes, it migrates back into canals and streams and into the Mekong again (Ref. 37770). Often used as a pituitary donor for artificial propagation in aquaculture. Escapees from culture installations have become established in rivers and form the basis for capture fisheries on several Southeast Asian islands (Ref. 1739). Useful in cropping excessive vegetation in reservoirs (Ref. 2686). Used for lap pa (in the preparation of which the numerous small bones are ground fine) or grilled or used to make som pa. Usually marketed fresh and occasionally seen in the aquarium trade (Ref. 12693). A specimen measuring 45 cm TL (2,100 g) was reportedly caught from Dan Tchang Reservoir, Thailand on 8 July 2003 (Jean-Francois Helias, pers. comm., FISHING ADVENTURES THAILAND [mailto:fishasia@ksc.th.com]).

34. *Puntioplites falcifer*

Classification

Order: Cypriniformes

Family: Cyprinidae

Etymology: Puntioplites: Latin, punctum = point, the point of the sword + Greek, hoplon = weapon (Ref. 45335).

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong basin

Size

Max length: 40.0 cm male/unsexed; (Ref. 37770)

Distribution

Last simple anal ray osseus and serrated posteriorly; pale orange anal, pelvic and dorsal fins; last simple dorsal ray reaching the caudal fin in adults and with 28-36 serrae (in specimens 6-15 cm SL) (Ref. 27732); serrated anal spine; silvery body coloration (Ref. 12693).

Biology

Inhabits large upland rivers. Seems to avoid standing water. Little is known about its biology. Like other members of the genus, it probably feeds mainly on plant matter and occasionally on insects and insect larvae (Ref. 12693). Also reported to prefer deep pools in the river and to migrate into streams, canals and lakes during the

flood season. Migrates in large schools (Ref. 37770). Migrates together with Cosmochilus harmandi, Cirrhinus spp., Labeo chrysophekadion and Bangana sp (Ref. 37770). Marketed fresh (Ref. 12693).

35. *Thynnichthys thynnoides*

Classification

Family: Cyprinidae

Etymology: Thynnichthys: Greek, thynnos = tunna + Greek, ichthys = fish (Ref. 45335).

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Borneo (Ref. 43281). Reported from the Maeklong river (Ref. 26336)

Size

Max length: 25.0 cm TL male/unsexed; (Ref. 4792)

Description

No Lips; plain silvery body (Ref. 43281).

Biology

Occurs in large rivers, canal, oxbows and floodplains. Microphagous, feeds mainly on phytoplankton and periphyton with lesser amounts of bottom algae and small zooplankton. Migrates for spawning to the floodplains when water level is high. Young of the year are caught as they begin to return to the rivers in October. In the Tonle Sap, adults make nearly all of the October catch, with larger and larger

proportions of young in subsequent months. Use to make prahoc and nuoc mam (Ref. 12693).

36. *Puntioplites proctozysron*

Classification

Family: Cyprinidae

Environment

Freshwater; benthopelagic; potamodromous (Ref. 51243). Tropical

Distribution

Asia: Maeklong, Chao Phraya and Mekong basins and Malay Peninsula.

Size

Max length: 30.0 cm (Ref. 4792)

Description

Body color brownish with reticulated dark scale edges; 17-22 dorsal spine serrations; serrated anal spine; very dark median fins, never orange (Ref. 12693). Last simple dorsal ray not reaching caudal fin in adults; dark grey anal, caudal and dorsal fins (Ref. 43281). Origin of pelvic fin opposite to origin of dorsal fin; lateral line decurved and extending to middle of caudal peduncle (Ref. 45563).

Biology

Inhabits large, slow flowing rivers (Ref. 43281). Commonly found in standing and slowly moving water of streams, canal, ditches and reservoirs. Moves into flooded forests and marshes during high water periods. Usually occurs around submerged aquatic or inundated terrestrial vegetation where it consumes some algae

but mostly insects and zooplankton. Larger fish are marketed fresh while smaller ones are used to make prahoc along the Tonlé Sap, Cambodia (Ref. 12693). Reaches more than 30 cm in length (Ref. 4792).

37. *Mystacoleucus marginatus*

Classification

Family: Cyprinidae

Etymology: Mystacoleucus: Greek, mystax = whiskered, used by Belon in 1553 to describe all fishes with whiskers + Greek, leykos = white (Ref. 45335).

Environment

Freshwater; benthopelagic. Tropical; 22°C - 27°C (Ref. 2059)

Distribution

Asia: Mekong, Chao Phraya and Meklong basins; also from the Malay Peninsula, Borneo, Sumatra and Java. Recorded from Myanmar (Ref. 4832).

Size

Max length: 20.0 cm (Ref. 7050); common length: 10.0 cm (Ref. 12693)

Description

Anal fin with a convex distal margin; dorsal fin with black anterior and distal margins; a black distal margin on the caudal fin; most scales with a black crescent-shaped mark on their base (Ref. 27732).

Biology

Found at bottom depths of rivers and streams. Inhabits areas with sand or pea-gravel from small streams to large rivers (Ref. 12693). Usually found in streams with clear and moving waters (Ref. 27732). Breeds when water levels begin to rise, but whether it leaves permanent water or not is unknown. Occasionally seen in markets (Ref. 12693)

38. *Systomus orphoides*

Classification

Order: Cypriniformes

Family: Cyprinidae

Environment

Freshwater; benthopelagic; Tropical

Distribution

Asia: locality is ‘Java, Indonesia’, but this species is currently considered to have a wide range extending eastwards from northern India and Myanmar via

Thailand, Laos, and Cambodia as far as Vietnam and to the south through Peninsular Malaysia and the Greater Sunda Islands of Borneo, Sumatra, and Java. https://www.seriouslyfish.com/species/systomus-orphoides/It thus occurs throughout the great Mae Klong, Chao Phraya and Mekong drainages as well as many smaller river basins, lakes and reservoirs. More recently it's recorded close to Chennai in Tamil Nadu state, southern India but it remains to be seen whether it's more widely-distributed than previously thought or has been introduced in some way (Knight and Rema devi 2010).

Size

Max length: 200 – 250 mm.

https://www.seriouslyfish.com/species/systomus-orphoides/

Description

Reference photo in https://www.seriouslyfish.com/species/systomus-orphoides/

Biology

Mostly inhabits flowing tributaries, large streams, and their surrounding floodplains. he fish move into inundated agricultural areas and forests during the wet season to feed and spawn, returning to the rivers as the water begins to recede. It's also found in some larger rivers and permanent water bodies including the highly seasonal Tonlé Sap system in Cambodia.

39. *Barbonymus schwanenfeldii*

Classification

Family: Cyprinidae

Environment

Freshwater; benthopelagic; pH range: 6.5 - 7.0; potamodromous (Ref. 51243). Tropical; 22°C - 25°C (Ref. 13371)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Borneo.

Size

Max length: 35.0 cm (Ref. 30857); common length: 20.0 cm (Ref. 4835)

Description

Dorsal spines (total): 3; Dorsal soft rays (total): 8; Anal spines: 3; Anal soft rays: 5. Distinguished from other species of the genus in having a red dorsal fin with a black blotch at the tip, red pectoral, pelvic and anal fins, red caudal fin with white margin and a black submarginal stripe along each lobe, and 8 scale rows between dorsal-fin origin and lateral line (Ref. 27732). Large individuals silvery or golden yellow in life with its dorsal fin red and caudal fin orange or blood-red (Ref. 2091)

Biology

Found in rivers, streams, canals and ditches (Ref. 12693). Occurs in medium to large-sized rivers and enters flooded fields (Ref. 12975). In east Kalimantan, Indonesia a temperature range of 20.4-33.7°C was recorded for this species (Ref. 6129). Largely herbivorous, consuming aquatic macrophytes and submerged land plants, as well as filamentous algae and occasionally insects (Ref. 12693). Also feeds on small fishes (Ref. 12693), worms and crustaceans (Ref. 7020). Usually marketed fresh (Ref. 12693).

40. *Osteochilus vittatus*

Classification

Family: Cyprinidae

Etymology: Osteochilus: Greek, osteon = bone + Greek, cheilos = lip (Ref. 45335).

Environment

Freshwater; benthopelagic; pH range: 6.5 - 7.0; potamodromous (Ref. 51243); Tropical; 22°C - 26°C (Ref. 2059)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java and Borneo (Ref. 27732). Reported from Salween and Maeklong basins (Ref. 26336); China (Ref. 9671); and lower Myanmar (Ref. 4832).

Size

Max length: 32.0 cm SL male/unsexed; (Ref. 7050); common length: 20.0 cm SL male/unsexed; (Ref. 2686)

Description

Dorsal soft rays (total): 17-19; Anal soft rays: 8. Distinguished from the other species of the genus in having 12-18 branched dorsal rays; 6-9 rows of spots along scale rows (not always distinct), and a large round blotch on the caudal peduncle (Ref. 27732). No black midlateral stripe; sometimes with a spot above a pectoral fin (Ref. 12693).

Biology

Adults occur in all type of habitats, but usually associated with large streams with slow current and muddy to sandy substrate (Ref. 27732). They migrate from river to flooded areas during the onset of the flood season and returns to river habitats at the end of that period (Ref. 37770). Juveniles are usually seen first in August; they move back to permanent water as flooded lands dry up. Back in the rivers they are attached to brush piles, tree roots and other solid objects (Ref. 12693). Adults feed on roots of plants (*Hydrilla verticillata*), unicellular algae and some crustaceans. Most abundant fish in Nam Ngum reservoir where it is captured with large dip nets set on rafts. Good flesh but bony and used for lap pa or grilled (Ref. 6459). Marketed fresh or used to make prahoc (Ref. 12693).

41. *Hampala dispar*

Classification

Family: Cyprinidae

Etymology: Hampala: Local name from Java

Environment

Tropical

Distribution

Asia: endemic to the Mekong basin.

Size

Max length :35.0 cm (Ref. 30857)

Description

Has a round midlateral blotch under the dorsal-fin origin in adults and a plain grey caudal fin (Ref. 27732); juveniles lack black teardrop-shaped marking on cheek; barbel always shorter than eye width (Ref. 12693).

Biology

Occurs in slowly moving or standing water habitats (Ref. 12693). Encountered also in rapid-running mountain streams of the middle Mekong (Ref. 12975). Common in impoundments, with small individuals frequenting areas of dense vegetation. Feeds mainly on prawns, crabs, and shrimps, along with some insect

larvae and some fish as well. Breeds at the start of the rainy season and the young are found in seasonally flooded habitats in June. Marketed fresh (Ref. 12693).

42. *Rasbora tornieri*

Classification

Family: Cyprinidae

Etymology: Rasbora: Rasbora, an Indian word for a fish, also used in Malay peninsula.

Environment

Freshwater; benthopelagic; Tropical

Distribution

Asia: Indochina (Ref. 12693, 30857), Malaysia and Indonesia.

Size

Max length: 17.0 cm (Ref. 30857)

Description

Dorsal soft rays (total): 9-10; Anal soft rays: 8; Vertebrae: 28 - 30. Brightly colored; body depth 4.2-4.6 times in SL; lateral line complete; 1 or 2 scale rows between lateral line and mid-ventral scale rows in front of pelvic fin (Ref. 12693).

Biology

Occur in streams, canals and ditches in lowland floodplains. Usually found right at the water surface and is easily recognized by the broad black margin on the bright yellow caudal fin. Feed on exogenous insects (Ref. 12693). Spawning sites are found in rivers and ponds (Ref. 33813). Mature adults probably breed during the rainy season. Occasionally seen in fish markets (Ref. 12693)

43.*Osteochilus vittatus*

Classification

Family: Cyprinidae

Etymology: Osteochilus: Greek, osteon = bone + Greek, cheilos = lip (Ref. 45335).

Environment

Freshwater; benthopelagic; pH range: 6.5 - 7.0; potamodromous (Ref. 51243); Tropical; 22°C - 26°C (Ref. 2059)

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java and Borneo (Ref. 27732). Reported from Salween and Maeklong basins (Ref. 26336); China (Ref. 9671); and lower Myanmar (Ref. 4832).

Size

Max length: 32.0 cm SL male/unsexed; (Ref. 7050); common length: 20.0 cm SL male/unsexed; (Ref. 2686)

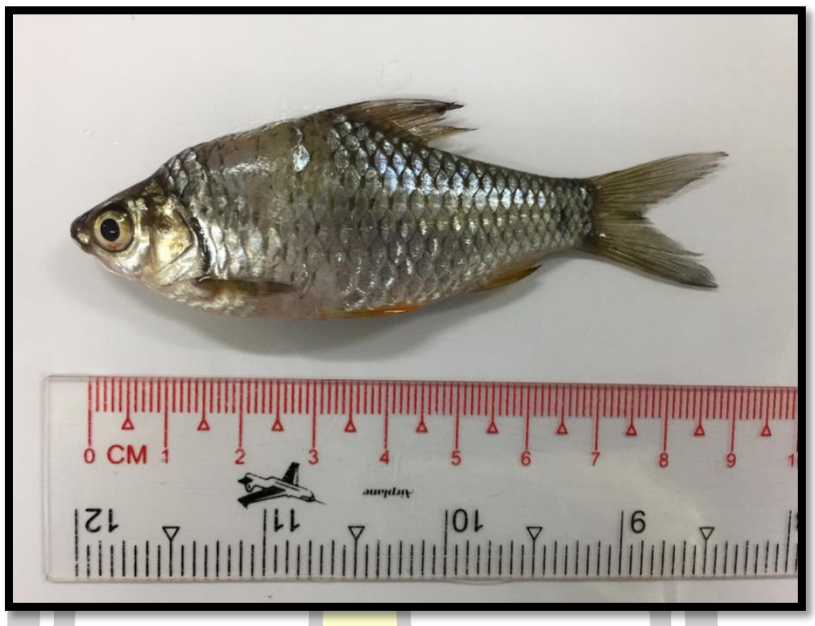
Description

Dorsal soft rays (total): 17-19; Anal soft rays: 8. Distinguished from the other species of the genus in having 12-18 branched dorsal rays; 6-9 rows of spots along scale rows (not always distinct), and a large round blotch on the caudal peduncle (Ref. 27732). No black midlateral stripe; sometimes with a spot above a pectoral fin (Ref. 12693).

Biology

Adults occur in all type of habitats, but usually associated with large streams with slow current and muddy to sandy substrate (Ref. 27732). They migrate from river to flooded areas during the onset of the flood season and returns to river habitats at the end of that period (Ref. 37770). Juveniles are usually seen first in August; they move back to permanent water as flooded lands dry up. Back in the rivers they are attached to brush piles, tree roots and other solid objects (Ref. 12693). Adults feed on roots of plants (*Hydrilla verticillata*), unicellular algae and some crustaceans. Most abundant fish in Nam Ngum reservoir where it is captured with large dip nets set on rafts. Good flesh but bony and used for lap pa or grilled (Ref. 6459). Marketed fresh or used to make prahoc (Ref. 12693).

44.*Mystacoleucus marginatus*



Classification

Family: Cyprinidae

Etymology: Mystacoleucus: Greek, mystax = whiskered, used by Belon in 1553 to describe all fishes with whiskers + Greek, leykos = white (Ref. 45335).

Environment

Freshwater; benthopelagic. Tropical; 22°C - 27°C (Ref. 2059)

Distribution

Asia: Mekong, Chao Phraya and Meklong basins; also from the Malay Peninsula, Borneo, Sumatra and Java. Recorded from Myanmar (Ref. 4832).

Size

Max length: 20.0 cm (Ref. 7050); common length: 10.0 cm (Ref. 12693)

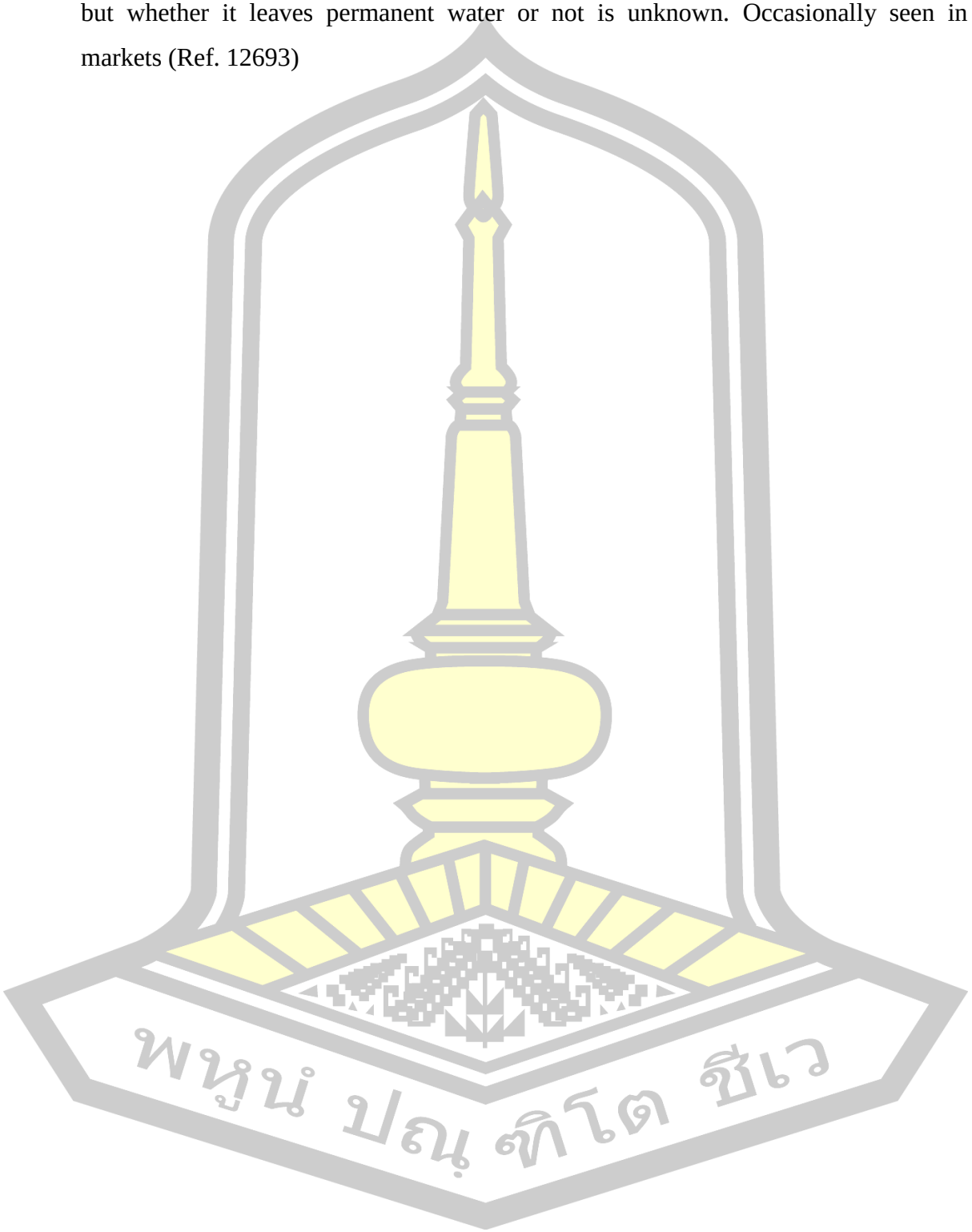
Description

Anal fin with a convex distal margin; dorsal fin with black anterior and distal margins; a black distal margin on the caudal fin; most scales with a black crescent-shaped mark on their base (Ref. 27732).

Biology

Found at bottom depths of rivers and streams. Inhabits areas with sand or pea-gravel from small streams to large rivers (Ref. 12693). Usually found in streams

with clear and moving waters (Ref. 27732). Breeds when water levels begin to rise, but whether it leaves permanent water or not is unknown. Occasionally seen in markets (Ref. 12693)



BIOGRAPHY

NAME	Miss Ei Ei Phyo Myint
DATE OF BIRTH	11 August 1985
PLACE OF BIRTH	Yangon
ADDRESS	27, A(1), Shwe Pinlon Villa, Rose Park 3, North Dagon Township, Yangon, Myanmar
POSITION	Assistant Lecturer
PLACE OF WORK	Department of Biochemistry, University of Medicine Taunggyi, Taunggyi, Myanmar
EDUCATION	2008 M.B.,B.S (Institute of Medicine 2) Yangon, Myanmar 2015 M.Sc (Medical Biochemistry and Molecular Biology) Mahidol University, Bangkok, Thailand 2018 Ph.D. (Health Sciences International Program) Mahasarakham University, Thailand
Research grants & awards	1. Scholarship for M.Sc (Medical Biochemistry and Molecular Biology, Mahidol University,Bangkok, Thailand) supported by China Medical Board 2. Outstanding Oral Presentation Award at International Bioscience Conference 3.Scholarship from Joint Fellowship Programme for Doctoral Students under Thai – Swedish Trilateral Cooperation
Research output	Oral Presentation Award for the presentation on the topic "Protective Role of N-Trans-feruloyltyramine in Hydrogen Peroxide-induced Cell Death" at the International International Bioscience Conference