CHAPTER 1

INTRODUCTION

In northern Thailand, the minute intestinal trematodes in the Family Heterophyidae, especially *Haplorchis taichui* Witenberg, 1930 and *H. pumilio* Looss, 1896, are major zoonotic parasites infecting humans and animals (Chai *et al*., 2005). These parasites have a complex aquatic life cycle, by using various species of freshwater snails and cyprinoid fishes as first and second intermediate hosts, respectively, with fish-eating mammals as definitive host (Pearson, 1964; Olsen, 1974; Fried *et al*., 2004; Faltýnková, 2005; Díaz *et al*., 2008; Uthpala *et al*.,2010). The World Health Organization (WHO) recently reported that there are over 18 million peoples around the world have already been infected with these trematodes. Now, Heterophydiasis becomes a major public health threat in South-East Asia especially, Thailand, Laos and Vietnam (Radomyos *et al*., 1984; Sukontason *et al*., 1999; Sohn and Chai, 2005; Chai *et al*.,2005).

Several epidemiological investigations of trematode infections in Thailand have shown high prevalence of heterophyid trematodes infections. The freshwater fishes from Mae Sa stream, Chiang Mai province were found to be mainly infected with four heterophyid trematode species including *Haplorchis* sp., *Haplorchoides* sp., *Centrocestus caninus* and *Stellantchasmus falcatus* (Wongsawad *et al*., 2004). The metacercarial stage of *H. taichui*, had high prevalence of infection in cyprinoid fishes collected from Chiang Mai Province (Boonchot and Wongsawad, 2005;

Kumchoo *et al*., 2005). Furthermore, Nithikathkul and Wongsawad (2008) reported a high prevalence of *H. taichui* and *Haplorchoides* sp. (83.90%) in the same area.

Various reports have been indicated the several species of heterophyid trematodes such as *H. taichui, H. pumilio, Centrocestus formosanus*, *Procerovum varium*, *Haplorchoides* sp. and *Stellantchasmus falcatus* develop from pleurolophocercous and parapleurolophocercous cercariae (Malek, 1922; Umadevi and Madhavi, 2000; Umadevi and Madhavi, 2006; Díaz *et al*., 2008; Skov *et al*., 2009).

More than 100 species of snails are known as an intermediate hosts for trematodes, especially the snail family Thiaridae, which harbor the larvae of intestinal trematodes, including *H. taichui* and *H. pumilio* (Subba, 1993).

The traditional methods to detect cercarial infections in snails are usually performed by exposing the snails to light and/or by dissection, so that the cercariae might be obviously observed. This methods are difficult to detect specific at the specific larval stage because the similar morphological traits may be exhibit which closely related to other trematodes (Pearson and Ow-Yang, 1982).

Because of their similar morphological appearances, a DNA specific primer for trematode diagnosis is necessary to aid in determination epidemiological occurrence of each trematode species. Various PCR methods have been developed to detect of trematode species in the intramolluscan stages (sporocyst, redia, and cercaria) namely, Amplified Fragment Length Polymorphism (AFLP), High Annealing Temperature–Random Amplified Polymorphic DNA (HAT-RAPD) and Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) (Barber *et al*., 2000; Lchikawa and Itagaki, 2010). The development of

specific DNA probes for *H. taichui* and *H. pumilio* detection has been rarely successful. Wongsawad *et al.* (2009a) and Wongsawad *et al.* (2009b) developed a DNA specific primer for *H. taichui* by using HAT-RAPD method for precise detection in the intermediate and definitive hosts. The PCR-RFLP utilizes mitochondrial cytochrome c oxidase subunit I (mtCOI) gene sequence marker to distinguish *Opisthorchis viverrini* from *H. taichui* (Thaenkham *et al*., 2007). Van *et al*. (2009) used ITS-2 gene sequencing to separate the larval and adult stages of *H. taichui* and *H. pumilio*. The ITS-2 gene was used as a shortcut tool to get the quick and accurate results, but gene conserving (strictly precise in closely related species) is lower than in those mitochondrial genomes such as the mtCOI gene. However, the identification such trematode parasites using PCR-RFLP of mtCOI gene is a time-consuming and costly.

Hence, the aims are to design a pair of DNA specific primers of the mtCOI gene to detect *H. taichui* and *H. pumilio* infection in freshwater snails. The DNA specific primer from this gene expected to be accurate, less time –consuming and giving higher specificity than previous reports. The examination of the phylogenetic relationship of heterophyid trematodes using mtCOI sequence data will be conducted. The distribution of both trematode species using of Geographic Information System (GIS) will be investigated to gain information on the distribution of these parasitic infections. Moreover, this will be a first step or fundamental providing new information on the latest distribution of trematode infection which will be useful for the development of the effective control measures in northern Thailand.

Research Objectives

1. To investigate the distribution and compare the prevalence of *Haplorchis taichui* and *H. pumilio* cercariae infected snail hosts from water resources in northern Thailand.
2. To develop DNA specific primer for a *H. taichui* and *H. pumilio* from mitochondrial cytochrome c oxidase subunit I (mtCOI) for identification in snail intermediate hosts.
3. To determine the phylogenetic relationship of heterophyid trematodes based on sequencing of mtCOI.