

CHAPTER 6

CONCLUSION

Snail-borne zoonotic intestinal trematodes are recognized as an important group of livestock and human pathogens. The number of people infected with the intestinal trematodes was estimated to be over 18 millions. Several species of trematodes in Heterophyidae family especially, *Haplorchis taichui* and *H. pumilio* are the minute intestinal parasite of carnivores, piscivorous animals and bird, including human, which are widely distribution in East and Southeast Asia. They have a completed life cycle with many species of the freshwater snail as their first intermediate host for producing the number of trematode distribution. Hence, this work was performed for investigating the cercarial infections in the freshwater snails and developed the DNA specific primer of *H. taichui* and *H. pumilio* for describing the geographic distribution of this trematode. Moreover, the phylogenetic relationship of heterophyid trematode was also analyzed based on the mtCOI sequences.

For the cercarial investigations, a total number of 4,533 snail individuals were infected to 14 cercarial types. The overall prevalence of cercarial infection was 14.65%. The parapleurolophocercous cercaria Type I was extensively distributed, as the result, it can be found in all provinces and low specificity for infection in the intermediate host snails, while some cercarial types were found in only one. This section of the study indicated the distribution of the larval stage of trematode in the North of Thailand. Moreover, this report can conclude that the freshwater snails can be considered as an important determinant to monitor the biomedical and veterinary

public health which can be applied to the prevention, management, and epidemiological control programs.

The specific primer of *H. taichui* and *H. pumilio* were established using mtCOI gene, which is can highly copy and more conserve than the nuclear DNA. The sequence of the both primer were shown as follows; HT-F (5' GTT-TGG-TTA-TGG-GGG-TTT-AGT-TCT-T 3'), HT-R (5' AAC-CTT-TAT-ACC-TGT-GGG-GAC-T 3') for *H. taichui* (anticipate product size 160 bp), HP-F (5' GGA-TGT-AAA-GAC-GGC-TGT-GTT-CTT-C 3'), and HP-R (5' TAG-GAT-CTC-AAA-ATC-GTC-TA 3') for *H. pumilio* (anticipate product size 125 bp). For the sensitivity tests, the *H. taichui* specific primer can be amplified the minimum concentration of DNA template, that was 0.45 ng/μl., while the *H. pumilio* specific primer can amplified at the minimum DNA template concentration at 1.18 ng/μl. In the study with specificity proof, both of specific primers were tested for ability and specific amplification with 8 trematode species and snail tissues, which have been reported as the widely distributed and high prevalence of the infection in the North of Thailand as they have been reported frequently in a past decade. The result showed that, a 160 bp and 125 bp were amplified as well as give the positive result in only *H. taichui* and *H. pumilio*, respectively, and there were not cross reaction with the other trematodes.

For the molecular identification of *H. taichui* and *H. pumilio* infected in the snails using multiplex PCR reaction. It can be indicated the snail in Thiaridae family are served as an intermediate host of *H. taichui* (*M. tuberculata*, *T. granifera* and *Thiara scabra*) and *H. pumilio* (*M. tuberculata* and *T. granifera* only). In addition, *H. taichui* are widely distributed in the North of Thailand, but *H. pumilio* were found in the lower part of the North of Thailand.

Regarding the phylogenetic relationship of heterophyid trematodes, the mtCOI sequences can demonstrated the relation of them. The phylogenetic and systematic of heterophyid trematodes can be determined by a molecular approach using mtCOI for DNA barcoding. Three group were defined according systematic criteria.

In summary, the study provides the information about the diversity of the intermediates hosts, prevalence and distribution of *H. taichui* and *H. pumilio* in the freshwater snails in the North of Thailand by morphological and PCR-based methods. Therefore, it can be stated that the study of cercarial state infection is very important. The necessary control measures for species is very important for human health, so this requires the identification of the several stages of trematode, the information of the life cycle, and the way of distinguishing the different parasites infecting the same host. Therefore, it is needed to identify the cercarial stage of all the trematodes, even of those which do not take in the big part, because of the possibility of competition for the same intermediate hosts. The authors expect that the results of this study will help to stimulate the undertaken of the further comparative studies, employing larger sampling schemes and involving more comprehensive evaluation of various aquatic for the trematode infection in the intermediates host.