CHAPTER 6

Conclusion

This study focused on the occurrence and identification of echinostome metacercariae in freshwater snails in Chiang Mai province. The echinostome metacercariae detected were *Echinostoma revolutum* (Fröelich, 1802) Looss, 1899. *Filopaludina doliaris, F. sumatrensis polygramma, F. martensi martensi, Eyriesia eyriesi, Bithynia funiculate, B. siamensis siamensis* and *Clea helena* snails were found infected with *E. revolutum* metacercariae. The overall prevalence of infection was 24.2% (2,586/10,692), with the mean intensity of 16.2 metacercaria per snail. All localities showed natural infection of *E. revolutum* metacercariae in snails. This is the first report for metacercaria of *E. revolutum* in snail host, *C. helena*, and also confirmed that *Filopaludina* spp., *E. eyriesi* and *Bithynia* spp. act as the second intermediate host of *E. revolutum.*

The whole life cycle of *E. revolutum* has been studied and, at least 80-83 days are required from metacercaria to metacercaria. Based on experimental life cycle studies beginning with infected snails from epidemic areas, it is shown that the life cycle consists of eight stages, viz. adult, egg, miracidium, sporocyst, mother redia, daughter redia, cercaria and metacercaria. The first and second intermediate hosts are viviparid snail; *F. doliaris* and *F. martensi martensi*. The final host is the domestic chick (*G. gallus domesticus*).

Loop-mediated isothermal amplification (LAMP) assay was established to detect *E. revolutum*. The *E. revolutum* specific-LAMP assay was successful developed, which used a set of 6 specific designed LAMP primers. The LAMP assay specifically amplified *E. revolutum* and had a high level of sensitivity comparable with the conventional PCR. Furthermore, the applicability of this assay for detection of *E. revolutum* was evaluated by examining field samples of echinostome metacercariae

from naturally infected snails. The LAMP assay can be used to effectively amplify the target DNA of *E. revolutum* metacercariae. The LAMP assay developed in this study is applicable for detection and epidemiological surveys of this trematode.

DNA sequencing of the ITS2 and ND1 were used to determine the phylogenetic relationships of *E. revolutum* between an isolate from Southeast Asia with other isolates. The results revealed that *E. revolutum* and other *Echinostoma* species of "*revolutum*" group were aligned as a monophyletic group. This study provided the molecular evidence from ITS2 and ND1 sequences to assess the phylogenetic relationships of *E. revolutum* from Southeast Asia. Genetic characterizations of *E. revolutum* are useful to achieve the basic information necessary for the systematics of this parasite.