CONTENTS

	Page
Acknowledgement	d
Abstract in Thai	e
Abstract in English	h
List of Tables	
List of Figures	0
Statement of Originality	
Chapter 1 Introduction	1
1.1 Research Objectives	-2
Chantar 2. Litaratura ravious	
Chapter 2 Elterature reviews	5
2.1 Taxonomic information	3
2.2 Morphology of adult worm	3
2.3 Morphology of larval stages	5
2.4 Epidemiology	8
2.5 Life history	11
2.6 Clinical symptoms	14
2.7 Diagnosis	14
2.8 Treatments	
2.9 Prevention and control	
2.10 Molecular parasitology	16

CONTENTS (CONTINUED)

		Page	
Chapter 3	Materials and Methods	21	
3.1	Materials and research instruments	21	
3.2	Chemical reagents	22	
3.3	Parasitic materials	23	
3.4	Morphological study of adult worms	24	
3.5	The prevalence of adult worms infection	25	
3.6	The prevalence of cercarial infection of snail intermediate hosts	25	
3.7	Life history study	30	
3.8	Molecular biological study	35	
Chapter 4	Results	40	
4.1	Morphological description of adult	40	
4.2	The prevalence of adult worm infection	42	
4.3	The prevalence of cercarial infection of snail intermediate hosts	43	
4.4	Life history study	47	
4.5	Molecular biological study	68	
Chapter 5	Discussion	99	
Chapter 6	Conclusion	105	
References	มหาวทยาลยเชย	107	
List of pub	by Chiang Mai Un	120	
	ights, reser		

CONTENTS (CONTINUED)



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF TABLES

		uze
Table 3.1	Nucleotide sequences of each arbitrary primer were used individually	
	for HAT- RAPD PCR	36
Table 4.1	The cercarial types and prevalence of infection found in snails	46
Table 4.2	The worm recovery rates of F. gigantica adult worms in albino mice	
	and dwarf hamsters	63
Table 4.3	Average measurements of young to mature adult of F. gigantica in both	
	experimental hosts; albino mice and dwarf hamsters	65
Table 4.4	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPA-01 primer	69
Table 4.5	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPA-02 primer	70
Table 4.6	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPA-03 primer	71
Table 4.7	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPA-04 primer	72
Table 4.8	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPA-09 primer	73
Table 4.9	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPA-10 primer	74
Table 4.10	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-02 primer	75
Table 4.11	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-03 primer	76
Table 4.12	Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-04 primer	77

LIST OF TABLES (CONTINUED)

		Page
Table	4.13 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-05 primer	78
Table	4.14 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-06 primer	79
Table	4.15 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-07 primer	80
Table	4.16 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-08 primer	81
Table	4.17 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-09 primer	82
Table	4.18 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPN-10 primer	83
Table	4.19 Number of fragment generated and fragment size ranged of each	
	trematode samples based on HAT-RAPD profiles of OPP-11 primer	84
Table	4.20 Information of primers used and total band generated, number of	
	polymorphic including percent polymorphism of each primer	85
Table	4.21 Specification of <i>F. gigantica</i> specific primers designed by the	
	sequences of RAPD markers of OPN-09 primer	88
Table	4.22 The maximum identities and query coverage of <i>F. gigantica</i>	94
Table	4.23 The variables speciation of genetic diversity of F. gigantica	
	and <i>F. hepatica</i>	98

LIST OF FIGURES

		Page
Figure 2.1	Illustration demonstrated the morphological comparison between	
	Fasciola hepatica and F. gigantica	5
Figure 2.2	Illustration demonstrated the larval forms of the closely	
	related-species, Fasciola hepatica	7
Figure 2.3	Illustration demonstrated the generally life cycle of closely	
	related-species, Fasciola hepatica	11
Figure 2.4	Diagram demonstrated the structure of ITS region in ribosomal gene	
	complex	19
Figure 3.1	Map of 7 sampling sites were designed for the snail	
	intermediate hosts collection in Chiang Mai Province	26
Figure 3.2	Sampling site 1: Ping River located at Chiang Dao district of	
	Chiang Mai Province	27
Figure 3.3	Sampling site 2: Mae Ngad River located at Phrao district of	
	Chiang Mai Province	27
Figure 3.4	Sampling site 3: Mae Taeng River located at Mae Taeng district of	
	Chiang Mai Province	28
Figure 3.5	Sampling site 4: Ping River located at Mae Rim district of	
	Chiang Mai Province	28
Figure 3.6	Sampling site 5: Ping River located at Chom Thong district of	
	Chiang Mai Province	29
Figure 3.7	Sampling site 6: Mae Cham River located at Hod district of	
	Chiang Mai Province	29
Figure 3.8	Sampling site 7: Doi Tao Reservoir located at Doi Tao district of	
	Chiang Mai Province	30
Figure 3.9	The experimental snail intermediate host; L.auricularia rubiginosa	32
	p p	

LIST OF FIGURES (CONTINUED)

	P	age
Figure 3.10	Rice plant pot for used to encystmentation of metacercaria	33
Figure 3.11	The experimental hosts; Phodopus campbelli and Mus musculus	34
Figure 4.1	Illustration demonstrated the morphology of F. gigantica adults from	
	Bos taurus and Bubalus bubalis	41
Figure 4.2	SEM photographs demonstrated the morphology of F. gigantica adults	
	from Bos taurus and Bubalus bubalis	42
Figure 4.3	The prevalence of <i>F. gigantica</i> adults infection recovered in <i>Bubalus</i>	
	bubalis and Bos taurus of Chiang Mai Province	43
Figure 4.4	The total prevalence of cercariae infection of snails found in each	
	sampling sites	44
Figure 4.5	The prevalence of infection in each cercarial type found in snails	45
Figure 4.6	Illustration demonstrated the morphology of <i>F. gigantica</i> eggs	46
Figure 4.7	SEM photographs demonstrated the morphology of <i>F. gigantica</i> eggs	47
Figure 4.8	Illustration demonstrated the morphology of F. gigantica miracidium	48
Figure 4.9	SEM photographs demonstrated the morphology of <i>F. gigantica</i>	
	miracidium	49
Figure 4.10	Illustration demonstrated morphology of F. gigantica young sporocyst	50
Figure 4.11	Illustration demonstrated morphology of F. gigantica mature sporocyst	51
Figure 4.12	Illustration demonstrated the morphology of F. gigantica mother redia	52
Figure 4.13	Illustration demonstrated the morphology of F. gigantica daughter redia	53
Figure 4.14	Illustration demonstrated morphology of F. gigantica cercaria	54
Figure 4.15	Illustration demonstrated the drawing morphology of	
	F. gigantica cercaria	55
Figure 4.16	Illustration demonstrated the morphology of capsule of metacercaria	56
Figure 4.17	Illustration demonstrated the morphology of metacercaria	57

LIST OF FIGURES (CONTINUED)

		Page
Figure 4.18	Photographs demonstrated the different stages of eggs during the	
	incubation period	58
Figure 4.19	Illustration demonstrated the development of the larval stages	59
Figure 4.20	Photographs demonstrated the cercariae released outer layer	
	of the cyst and lost its tail	60
Figure 4.21	Photographs demonstrated the capsule of metacercaria	61
Figure 4.22	Infected liver of a dwarf hamster was severely destroyed	
	due to migration and penetration of worms	62
Figure 4.23	The worm recovery rates of F. gigantica adult worms in	
	albino mice and dwarf hamsters	62
Figure 4.24	Immature eggs were discovered in day 42 PI on uterus	
	of dwarf hamsters	64
Figure 4.25	Changes of morphological and average measurements of	
	F. gigantica recovered from dwarf hamsters	66
Figure 4.26	Changes of morphological and average measurements of	
	<i>F. gigantica</i> recovered from albino mice	67
Figure 4.27	HAT-RAPD profiles generated by OPA-01 primer	69
Figure 4.28	HAT-RAPD profiles generated by OPA-02 primer	70
Figure 4.29	HAT-RAPD profiles generated by OPA-03 primer	71
Figure 4.30	HAT-RAPD profiles generated by OPA-04 primer	72
Figure 4.31	HAT-RAPD profiles generated by OPA-09 primer	73
Figure 4.32	HAT-RAPD profiles generated by OPA-10 primer	74
Figure 4.33	HAT-RAPD profiles generated by OPN-02 primer	75
Figure 4.34	HAT-RAPD profiles generated by OPN-03 primer	76
Figure 4.35	HAT-RAPD profiles generated by OPN-04 primer	77
Figure 4.36	HAT-RAPD profiles generated by OPN-05 primer	78

r

LIST OF FIGURES (CONTINUED)

		Page
Figure 4.37	HAT-RAPD profiles generated by OPN-06 primer	79
Figure 4.38	HAT-RAPD profiles generated by OPN-07 primer	80
Figure 4.39	HAT-RAPD profiles generated by OPN-08 primer	81
Figure 4.40	HAT-RAPD profiles generated by OPN-09 primer	82
Figure 4.41	HAT-RAPD profiles generated by OPN-10 primer	83
Figure 4.42	HAT-RAPD profiles generated by OPP-11 primer	84
Figure 4.43	DNA profiles demonstrated that the expected approximately	
	fragments of 380 base pairs of F.gigantica-specific bands	86
Figure 4.44	Illustration demonstrated nucleotide sequences of	
	F.gigantica-Specific derived from HAT-RAPD markers	87
Figure 4.45	Illustration demonstrated the designed position of the 235 bp	
	nucleotide sequences of F. gigantica specific primer	88
Figure 4.46	Illustration demonstrated specificity test of	
	F. gigantica -specific primers	89
Figure 4.47	Illustration demonstrated the reaction sensibility of	
	F. gigantica -specific primers based on 10 folds serial dilution method	190
Figure 4.48	Illustration demonstrated the reaction specificity of	
	F. gigantica -specific primers	91
Figure 4.49	Illustration demonstrated the specific confirmation of F. gigantica	92
Figure 4.50	DNA profiles of ITS-2 region derived from BD2-R and 3S-F primer	93
Figure 4.51	Illustration demonstrated 533 ITS2 nucleotide sequences data of	
	F. gigantica	93
Figure 4.52	Phylogram derived from maximum likelihood analysis depicting	
	phylogenetic relationships of trematodes	96
Figure 4.53	Phylogram derived from an UPGMA analysis depicting phylogenetic	
	relationships of trematodes	97
	-5 -5 -5 -5 -5 -5 -5 -5	

ข้อความแห่งการริเริ่ม

- เป็นการศึกษาชีวประวัติของพยาธิใบไม้ F. gigantica ที่สมบูรณ์ครั้งแรกในสัตว์ทคลองใน ประเทศไทย
- 2) ข้อมูลของการติดเชื้อระยะตัวอ่อนในหอยที่เป็นโฮสต์กึ่งกลางของพยาธิใบไม้ F. gigantica

4)

- ไพรเมอร์แบบจำเพาะของพยาธิใบไม้ F. gigantica โดยใช้เทคนิค HAT-RAPD เป็นเครื่องมือ ใหม่สำหรับตรวจสอบตัวอ่อนของพยาธิใบไม้ F. gigantica ในหอยที่เป็นโฮสต์กึ่งกลาง
 - การวิเคราะห์ความสัมพันธ์เชิงวิวัฒนาการโดยใช้ลำคับนิวคลีโอไทค์ของส่วน ITS-2 เป็น เครื่องมือที่มีประสิทธิภาพในการระบุชนิดของพยาธิใบไม้ *F. gigantica*

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights_t reserved

STATEMENT OF ORIGINALITY

- 1) The first investigation of life history completely study of *F. gigantica* in experimental hosts in Thailand.
- 2) Detail of investigation of *F. gigantica* larval stages infections in snail intermediate hosts.
- 3) A specific primer of *F. gigantica* based on HAT-RAPD technique is the new tool for detection of *F. gigantica* larval stages in snail intermediate hosts.
- 4) The phylogenetic analysis based on ITS-2 sequence has been effective diagnostic tool for the identification of *F. gigantica*.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved