

## **APPENDICES**

## **APPENDIX A**

SEQUENCE OF MITROCHONDRIAL CYTOCHROM C OXIDASE GENE  
(MT COI) SUBJECTED FOR DESIGNED DNA SPECIFIC PRIMERS OF  
*Haplorchis taichui* AND *Haplorchis pumilio* AND GENERATE  
PHYLOGENETIC RELATIONSHIPS

**Sequences of mtCOI subjected for multiple alignment and designed DNA specific primers of *Haplorchis taichui* and *H. pumilio***

*Haplorchis taichui* (accession number KC404636)

TCGGGTCTAACTAAAGCCCTTTCGTAAGTGAGACACATTTGTAGGACGTT  
 AACAAATAAAGATTCCTTGTTTGGTTATGGGGGTTTAGTTCTTGCTATGTT  
 TTCTATAGTCTGTTTGGGGAGTGTTGTTTGGAGCTCATCATATGTTTATGGTT  
 GGGTTGGATGTTAAGACGGCTGTTTTTTTTAGTTCTGTGACTATGGTTATA  
 GGAGTCCCCACAGGTATAAAGGTTTTTTCTTGGCTGTATATGTTGGCGGGA  
 AGTCGGGGCCGGTTTTGGGATCCGATAATGTGGTGGATATTGGGTTTTATT  
 GTCCTATTTACTATCGGGGGGGTGACCGGGATTGTGTTGTCTTCTTCTATA  
 ATGGATACTTTGTTGCATGACACTTGATTTCGTAATAGCTCATTTTCATTAT  
 GTTCTTTCTTTAAA

*Haplorchis taichui* (accession number JN809909)

TTTGGTTTGGTATGGTTAGTCACATTTGTAGGACGTTAACAAATAAAGATT  
 CCTTGTTTGGTTATGGGGGTCAAGTTCTTGCTATGTTTTCTATAGTCTGTTT  
 GGGGAGTGTTGTTTGGAGCTCATCATATGTTTATGGTTGGGTTGGATGTTAA  
 GACGGCTGTTTTTTTTAGTTCTGTGACTATGGTTATAGGAGTCCCCACAGG  
 TATAAAGGTTTTTTCTTGGCTGTATATGTTGGCGGGAAGTCGGGGCCGGTT  
 TTGGGATCCGATAATGTGGTGGATATTGGGTTTTATTGTCTTATTTACTATC  
 GGGGGGGTGACCGGGATTGTGTTGTCTTCTTCTATAATGGATACTTTGTTG  
 CATGACCCATGGTTTA

*Haplorchis taichui* (accession number JN809893)

TTTGGTTTGGTATGTTGAGTCACATTTGTAGGACGTTAACAAATAAAGATT  
 CCTTGTTTGGTTATGGGGGTTTAGTTCTTGCTATGTTTTCTATAGTCTGTTT  
 GGGGAGTGTTGTTTGGAGCTCATCATATGTTTATGGTTGGGTTGGATGTTAA  
 GACGGCTGTTTTTTTTAGTTCTGTGACTATGGTTATAGGAGTCCCCACAGG  
 TATAAAGGTTTTTTCTTGGCTGTATATGTTGGCGGGAAGTCGGGGCCGGTT  
 TTGGGATCCGATAATGTGGTGGATATTGGGTTTTATTGTCTTATTTACTATC

GGGGGGGTGACCGGGATTGTGTTGTCTTCTTCTATAATGGATACTTTGTTG  
CATGACCCTTGGTTTA

*Haplorchis taichui* (accession number JN809875)

TTTGGTTTGGTATGGTGAGTCACATTTGTAGGACGTTAACAAATAAAGATT  
CCTTGTTTGGTTATGGGGGTTTAGTTCTTGCTATGTTTTCTATAGTCTGTTT  
GGGAGTGTTGTTGAGCTCATCATATGTTTATGGTTGGGTGGATGTTAA  
GACGGCTGTTTTTTTTAGTTCTGTGACCATGGTTATAGGAGTCCCCACAGG  
TATAAAGGTTTTTTCTTGGCTGTATATGTTGGCGGGAAGTCGGGGCCGGTT  
TTGGGATCCGATAATGTGGTGGATATTGGGTTTTATTGTCTTATTTACTATC  
GGGGGGGTGACCGGGATTGTGTTGTCTTCTTCTATAATGGATACTTTGTTG  
CATGACCCATGGTTTA

*Haplorchis taichui* (accession number EF055885)

TGTGCTGATTTTGCCGGGGTTCGGTATAGTGAGACACATTTGTAGGACGTT  
AACAAATAAAGATTCCTTGTTTGGTTATGGGGGTTTAGTTCTTGCTATGTT  
TTCTATAGTCTGTTTGGGGAGTGTTGTTTGGAGCTCATCATATGTTTATGGTT  
GGGTTGGATGTTAAGACGGCTGTTTTTTTTAGTTCTGTGACCATGGTTATA  
GGAGTCCCCACAGGTATAAAGGTTTTTTCTTGGCTGTATATGTTGGCGGGA  
AGTCGGGGCCGGTTTTGGGATCCGATAATGTGGTGGATATTGGGTTTTATT  
GTCTTATTTACTATCGGGGGGTGACCGGGATTGTGTTGTCTTCTTCTATA  
ATGGATACTTTGTTGCATGACACTTGGTTTTGGTTTA

*Haplorchis pumilio* (accession number KF044303)

TGTGACCTTCCTTTGTCATACCTTAATTGTCCTACATAAGACTAATAATTG  
ATTCCCTGTTTGGATATGGAGGTTTGGTTTTGGCTATGTTTGCTATAGTTTG  
TTGGGTAGTGTGGTTTGGAGCTCATCATATGTTTATGGTTGGCTTGGATGT  
AAAGACGGCTGTGTTCTTCAGTTCTGTA ACTATGATTATTGGGGTTCCTAC  
TGGGATAAAGGTTTTTTTCGTGGTTGTATATGTTAGCGGGGAGTCGTAGACG  
ATTTTGAGATCCTATTCTATGGTGGATATTAGGGTTTATTGTTTTGTTTACT  
ATAGGGGGTGTA ACTGGTATAATGTTGTCCGCATCTATAATGGATACTTTG  
CTTCATGATACTTGATTTGTAATTGCTCATTTTCATTATGTTCTTTCTTTAA

*Centrocestus caninus* (accession number KF044300)

TCATATCTGTATGACACTAACAAAAAAGATTCATTGTTTGGATATAGAG  
 GTTTGGTTCTGGCAATGTTTGCTATAGTTTGTTTAGGGAGAGTGGTTTGGG  
 CTCATCATATGTTTATGGTCGGGTTGGATTTGAAGACAGCTGTTTTTTTA  
 GGTCTGTAACATGATTATAGGGGTTCCCTACCGGTATAAAGGTTTTTTCTT  
 GATTGTATATGTTGGCTGGTAGACGCGGGCGTTTTTTGAGATCCGGTAATGT  
 GGTGAATTATAGGATTTATAGTATTATTCACGATAGGTGGTGTACGGGG  
 ATAATGTTGTCAGCTTCGATAATGGATACTTTGTTGCATGACACGTGGTTT  
 GTGATTGCGCATTTCATTATGTTCTTTCTTTAA

*Stellantchasmus falcatus* (accession number KF044301)

AGACATGTGTGTATGACGCTTACGAAAAATGATTCTCTTTTTGGTTACGGC  
 GGTCTCGTGCTTGCTATGTTTTCGATTGTGTGTCTTGGCAGAATTGTTTGGG  
 CTCATCATATGTTTATGGTGGGTTTGGATTTGAAGACTGCTGTGTTTTTTAG  
 CTCTGTAACATGATTATAGGGGTGCCGACGGGGATTAAGGTGTTTTTCATG  
 ATTGTACATGTTGGCGGGTAGCCGGGGGCGTTCTGAGACCCCGTTATGT  
 GGTGAATATTGGGTTTTATAGTCCTGTTTACTATAGGTGGTGTACTGGGG  
 TGGTTCTTTCTTCTTCTGTTATGGATACTTTAATGCATGATACTTGATTCTG  
 GATAGCTCATTTCATTATGTTCTTTCTTTAA

*Haplorchoides* sp. (accession number KF044302)

ATATACTGTACTACTTTGACTAAAAATGATTCTTTATTTGGTTATGGGGGG  
 TTGGTTTTTGCATGTTTGCTATAGTTTGTCTGGGTAGGGTTGTTTGGAGCCC  
 ATCATATGTTTATGGTTGGTTTAGATTTAAAGTCTGCGGTGTTTTTTAGTTC  
 TGTAACATGTTGATAAGGGTGCCTACAGGTATAAAGGTTTTTTCTTGACT  
 ATATATGTTGGCTGGTAGGAAGGGCCGTTTTTGAGATCCAATAATGTGGT  
 GGATTTTAGGATTTATAGTATTGTTTACAATAGGGGGTGTGACTGGAATTA  
 TGCTTTCTGCTTCAATGATGGATACGTTATTGCACGATACGTGGTTTGTTA  
 TAGCCCATTTTCATTATGTTCTTTCTTTAA

*Ganeo tigrinus* (accession number KF044307)

AAATTAAGTATCATGCATAAGCATATCTAATATAGAAGCAGAAAGCACAA  
TACCAGTAACTCCTCCAATCGTAAAAAGAAAAATAAAACCAACAATTCAC  
CACATAATAGGATCCCCAAAACGCATATACCTACCCCCAACATATACAA  
CCAAGAAAACACCTTAATACCGGTAGGAATACCAATAACCATAGTAACAG  
AACTAAAAAAAACAGCAGTCTTTAAATCCAAACCAATCATAAACATGTGG  
TGGGCCCAAACCTACCCTACCAAGACACACTATAGCCGCCATCGCAAAAAT  
CAACCCAAAATAACCAACAAAGAATCTTTTTTCGTAAGTCTCACACAAA  
TATGACTAACCACTCCAAACCCTGGCAATATCAAACATAAACCTCAGG

*Prostorhigenes majeedi* (accession number KF044308)

ATCATGCAACAAAGAATCCAAAACAGAAGCTGACAATACAACACCAGTT  
ACACCACCAATAGTAAACAAAAAATAAAACCCAAAACCCACAAAACCTA  
TAGGATTAGATAAACAACCACGACACCCACTAAGCATGTATAATCAAGAA  
AAAACCTTTTATACCCGTAGGAATACCAATAATCATAGTAATAGAACTAAA  
AAATATTGTAGTCTGCAAATCAAACCCAACAACAACATATGATGCCCCC  
AAACAACACTACCTAAACAAACTATAGAACCCATAGCAAAAACCATACCC  
AAATAACCAAAAACCCCCCTACCCCTACTCAGAGTACCACATATATGACC  
TACAATACCAAAAACCCAGGCAAAATCAAACATAAACCTCAGGATGCCCA  
AAAAA

*Fasciola gigantica* (accession number KF044304)

AATATCATGGGCAACGTATCCAAAGAGAAGCAGAAAGCATAATACCAGT  
AACCCACCAATAGTAAACAAAACAACAACCAACAATCCATCACACA  
ACAGGATCCCAAATACGAACAGAACTGCCACCTCCCAACATTATTAACCA  
AGAAAAAACCTTAATCCCCGTAGGAATACCAATAACCATAGTAACAGAAC  
TAAAAAAAACAGCAGTATGTACATCCAAACCCACCATAAACATATGATGA  
GCCCAAACAACACTACCCAAACATACTATAGCAGCCATAGCCAAAATAAG  
ACCATAATAACCAATAAAGAATCATTATTAGTCAAAGTCATACAAATAT  
GACTAATAACCCCAAACCCGGGCAAAATTAACATAAACCTCAGG

*Fischoederius elongatus* (accession number KF044305)

CCCCAAAAAACCAAGGTATCATGAACCATAGAATCCAACACACAAGAA  
 GATAGCACTATACCTGTCACACCTCCCATAGTAAACAAAATAATAAAACC  
 CATAATTCACCATAACAACAGGATCCCAAGCACGAACTCCAACACCACTCA  
 ACATATAACAACCAAGAAAACACCTTAATCCCTGTCGGTATACCTATCACC  
 ATCGACACAGAACTAAAAAACACAGATGTCTTCACATCTAACCCAACCAT  
 AAACATATGATGGGCCAAACAACACTACCTAAACAAACAATAGAAGCC  
 ATAGCAAATACTATACCATAGTAACCAAACAACGAATCTTGATTACTTAA  
 ACTCATACAAATATGTCTCACAGCCCCAAAACCAGGCAAATTAGCACAT  
 AAACCTCAGGATGCCCAAAAAAACC

*Orthocoelium streptocoelium* (accession number KF044306)

AAAAAAAAACCAAAGTATCATGCACCATAGAATCTAAAACACAAGAAGA  
 TAACACTATTCTGTTACACCACCCATAGTGAACAACACAATAAAACCCA  
 TAATTCACCATATTACTGGATCTCACATACGAACCCCCGTTCCACTTAACA  
 TATATAATCAAGAAAAAACCTTAATACCAGTTGGTATACCAATTACCATC  
 GACACAGAACTAAAAAACACAGATGTCTTCACATCTAAACCCACCATAAA  
 CATATGATGGGCTCAAACAACACTACCTAAACAAACAATCGAAGCCATAG  
 CGAACACTAAACCATAATAACCAAACAAGAATCCTGATTCTAACGCTC  
 ATACAAATATGACTCACAGCACCAAACCAGGTAAAATTAACACATAAAC  
 CTCAGGATGCCCAAAAA

*Metagonimus* sp. (accession number AF096232)

GTGCTTTTTGCCGGGGTTCGGTATAATTAGCCATATTTGTATGACTTTGAC  
 TAAAAATGATTCTGTTTCGGTTATGGGGGTTTGGTGTGGCTATGCTTGCGA  
 TAGTGTGCTTGGGCAGGGTTTTGAGCCCATCACATGTTTATGGTAGGTTTG  
 GATTTGAAGACAGCTGTTTTCTTTAGCGGTGACCATGATTATTGGGGTGCC  
 TACGGGTATAAAGGTGTCCTCCTGGCTGTATATTGGCAGGTAACCGAGGT  
 CGGTTTTGGGATCCTGTGATGTTGGTTGGATATTGGGGTTTTTGTCTCTTT  
 ACTATTGGGGGGGTTACTGGAATAATCCTTGTCCACCTCAATAATGGAAC  
 CCCTGTTTACATGACACTTGGTTTTGTTTATCCC

*Metagonimus yokogawai* (accession number AB470519)

GTGTTGATTCTCCCAGGGGTTTGGGATTATTAGGCATATATGCATGAACTC  
TGAATAAAAATGATTCCTTGTTTGGCTATGGCGGTTTAGTGTTGGCTATGC  
TTGCGATAGTGTGCTTGGGTAGTGTTGTTTGGGCGCATCACATGTTTATGG  
TGGGTCTGGATTTGAAGACGGCAGTCTTCTTTAGCTCGGTAACATGGTAA  
TTGGGGTGCCGACGGGTATAAAGGTGTTTTCTGGCTGTACATGTTGGCGG  
GTAGGCGAGGCCGCTTCTGAGACCCGGTGATGTGGTGGATACTTGGGTTT  
ATTGTCCTTTTTACTATTGGGGGGTCACTGGAATAATCTTATCGGCCTCC  
ATAATGGACACTCTGTTGCATGACACTTGGTTTGTGATAGCT

*Metagonimus takahashii* (accession number AF096231)

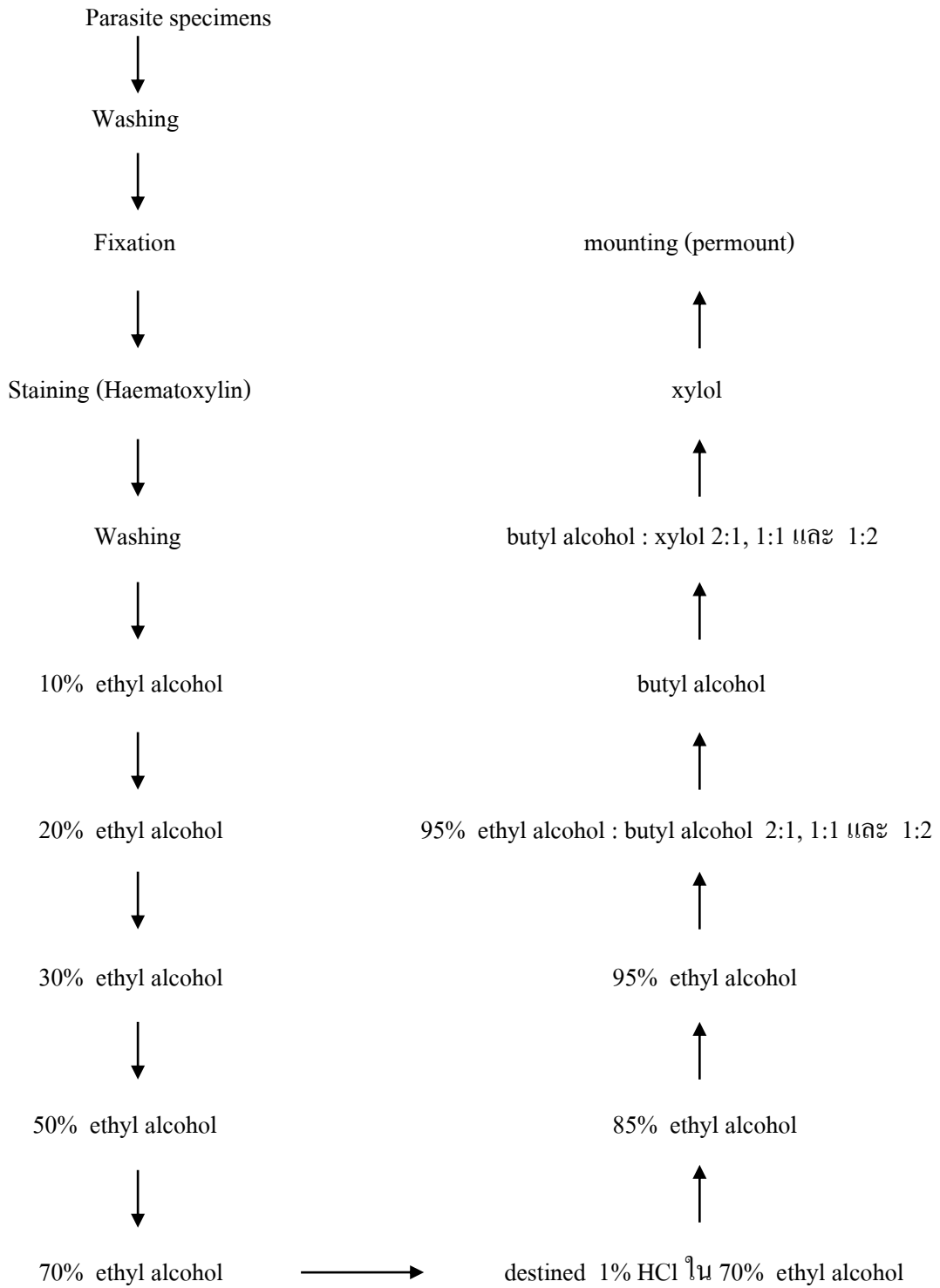
GTGTTGATTCTTCCTGGGTTTGGCATTATTAGGCATTATGTATGACTCTAA  
CTAAAAATGACTCTCTGTTTGGTTACGGCGGCTTAGTGCTGGCTATGCTCG  
CAATTGTATGCCTTGGTAGTGTCGTCTGGGCTCATCACATGTTTATGGTGG  
GTGGATGTGAAGACGGCAGTCTTCTTCAGTTCGGTGACTATGATAATCGG  
GGTACCGACGTATAAAGGTGTTTTCTTGGTTGTACATGTTGGCGGGTAGGC  
GGGGTCGGTTTTGAGATCCGGTGATGTGGTGGATTCTTGGATTCATTGTTC  
TTTTACCATTGGAGGGGTGCACTGGGATAATTTTATCGGCGCTCGATAAT  
GGATACTGCTGTTGCATGACACTTGGTTTGTATAGCTG



**APENDIX B**

**PROCESSES FOR PERMANENT SLIDE PREPARATION AND  
CHEMICAL REAGENTS**

### Processes for permanent slide preparations



**Chemical reagents****1. 1% Acid pepsin solution**

0.85% normal saline	99	ml
Pepsin powder	1	g
Hydrochloric acid	1	ml

**2. Heamatoxyline**

Distilled water	70	ml
Alcohol 95%	4	ml
Aluminium alum	3	g
Haematoxylin	0.6	g
Glycerine	15	ml
Methanol	15	ml

**3. Borax carmine**

Distilled water	25	ml
Alcohol 70%	50	ml
Borax	1	g
Carmine	1.5	g

**4. 4% formalin**

Distilled water	96	ml
Formaldehyde 40%	4	ml

**5. 5X Tris Boric acid EDTA (TBE) buffer**

Tris base	54	g
Boric acid	27.5	g
0.5 M EDTA (pH 8.0)	20	ml

Add distilled water to 1,000 ml and autoclave

## CIRRICULUM VITAE

**Name** Mr. Thapana Chontanarth

**Date of birth** 11 September 1985

### Education

2008 B.S. (zoology)

Chiang Mai University, Thailand

2010 M.S. (Biology)

Chiang Mai University, Thailand

2013 Ph.D. (Biodiversity and Ethnobiology)

Chiang Mai University, Thailand

### Publications

1. Chontanarth T. and Wongsawad C. 2010a. Prevalence of *Haplorchis taichui* in field-collected snails : A molecular approach. Korean J Parasitol. 48(1):343-6.
2. Chontanarth T. and Wongsawad C. 2010b. *Haplorchis taichui* infection of the freshwater snails and molecular identification. Trend Res Sci & Tec., 2(1): 7-

3. Chontanarith T. and Wongsawad C. 2013a. Epidemiology of cercarial stage of trematodes in freshwater snails from Chiang Mai Province. *Asian Pac Trop Biomed.*, 3(3): 237-243.
4. Chontanarith T. and Wongsawad C. 2013b. Prevalence of *Haplorchis taichui* infection in snails from Mar Taeng basin, Chiang Mai province by using morphological and molecular technique., *J. Yala Rajabhat University*, 1(1) (in press).

#### **Academic activities**

1. Reviewer of International Journal of Biology and Biological Sciences.
2. Reviewer of the Global Advances Research Journal of Geology and Mining Research.