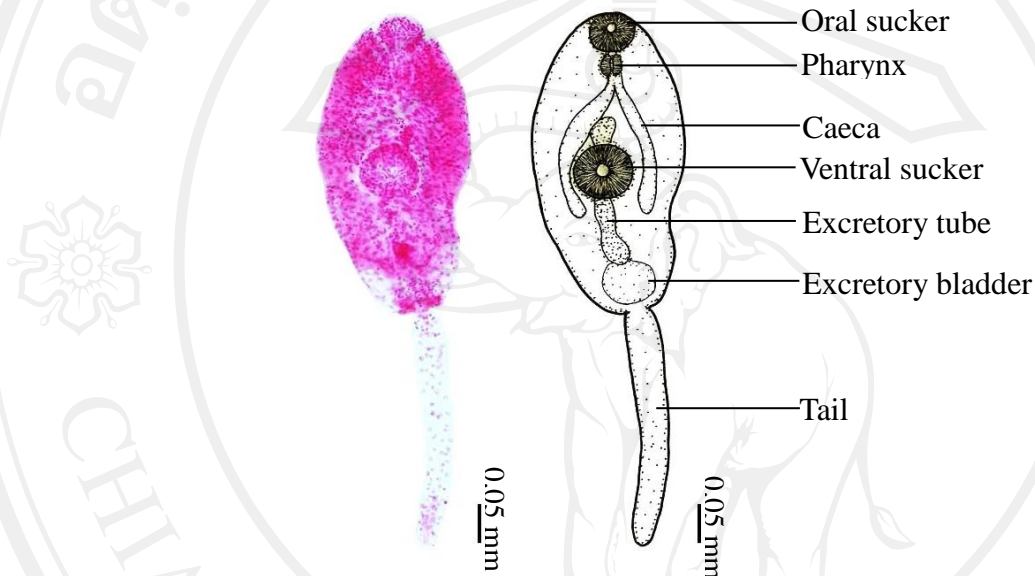


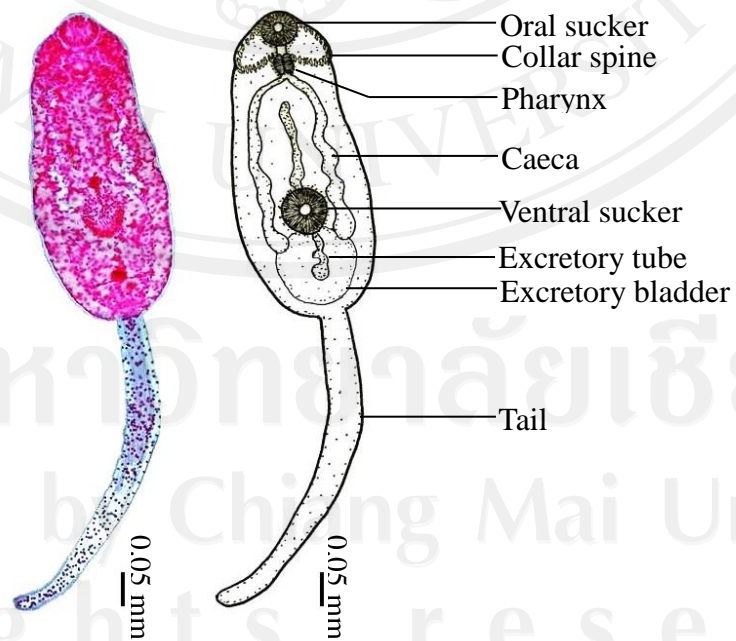
APPENDIX A

Cercarial types found in field-collected snails

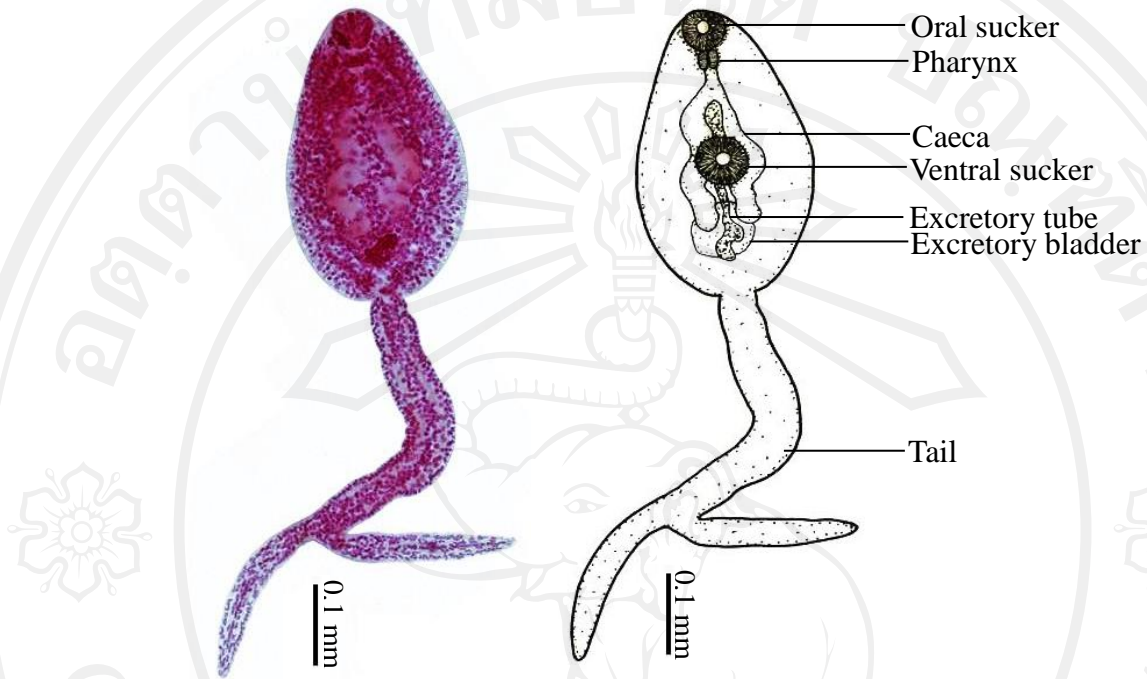
1. Distome cercaria



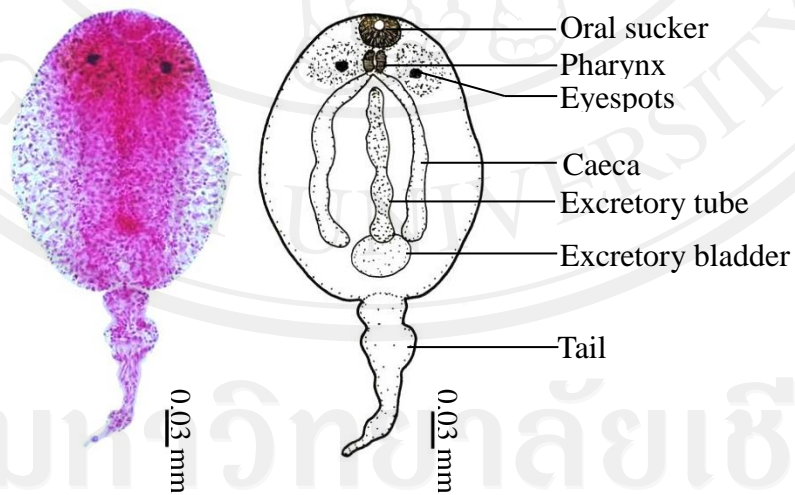
2. Echinostome cercaria



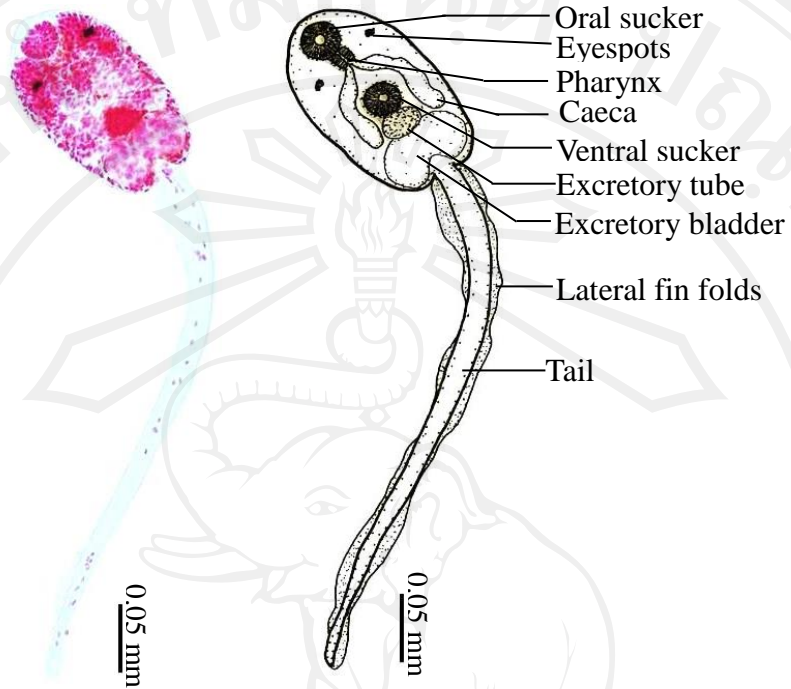
3. *Furcocercous cercaria*



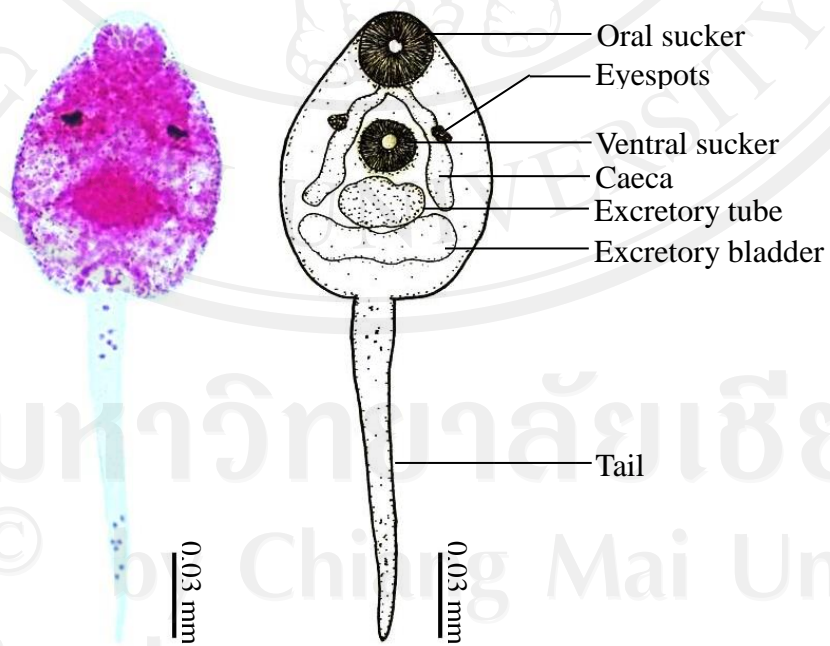
4. *Monostome cercaria*



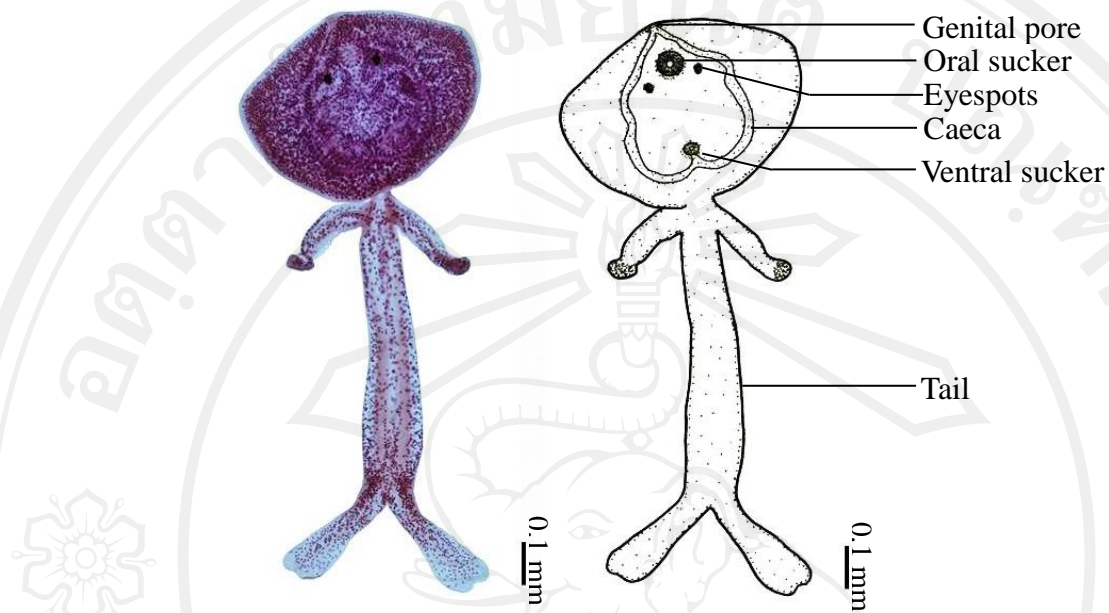
5. Parapleurolophocercous cercaria



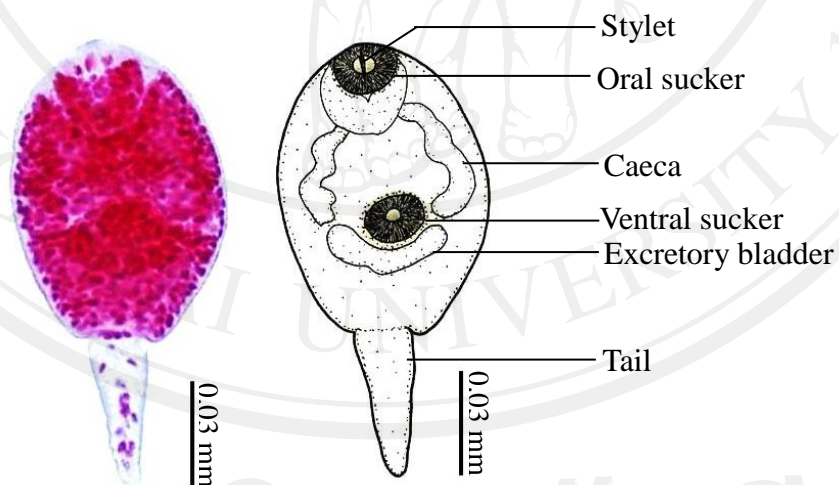
6. Pleurolophocercous cercaria



7. *Tranversotrema cercaria*



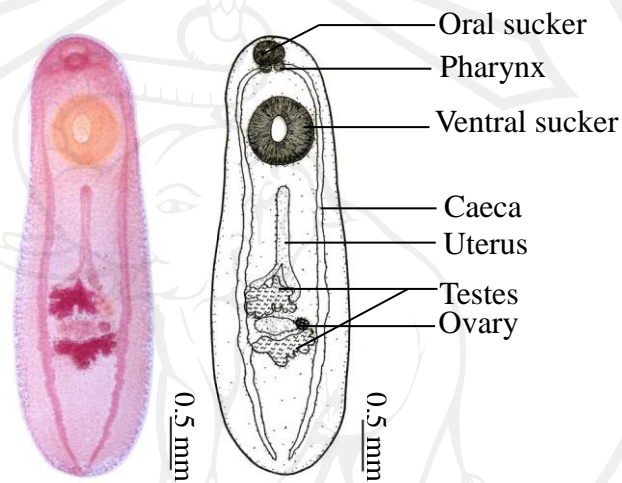
8. *Xiphidio cercaria*



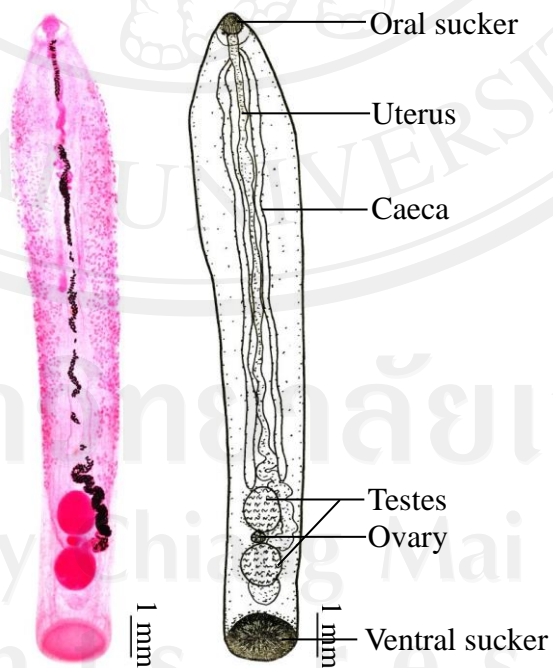
APPENDIX B

The related trematodes were compared

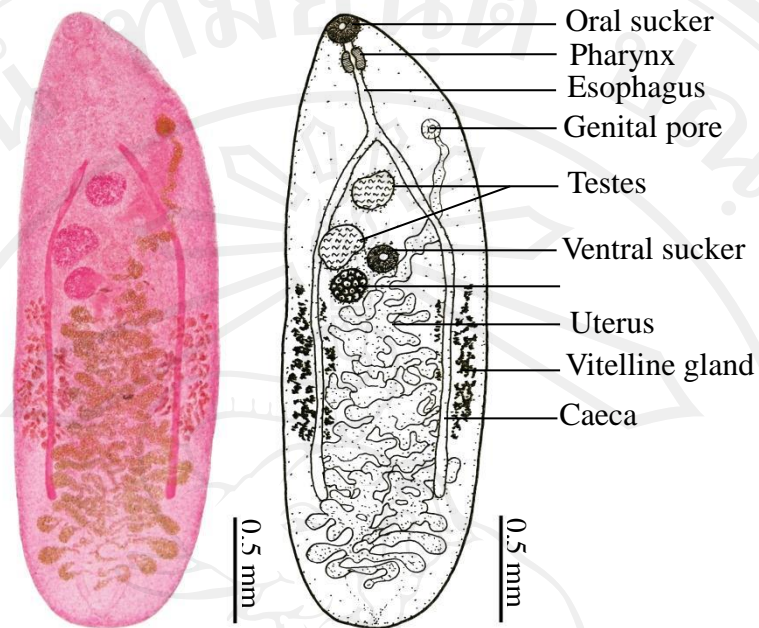
1. *Clinostomum philippinensis*



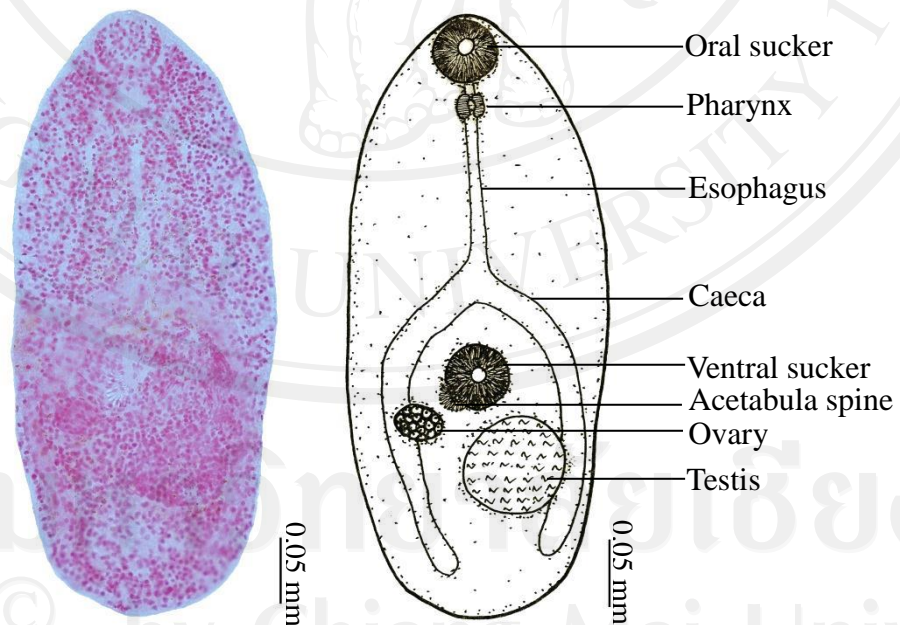
2. *Fischoederius elongatus*



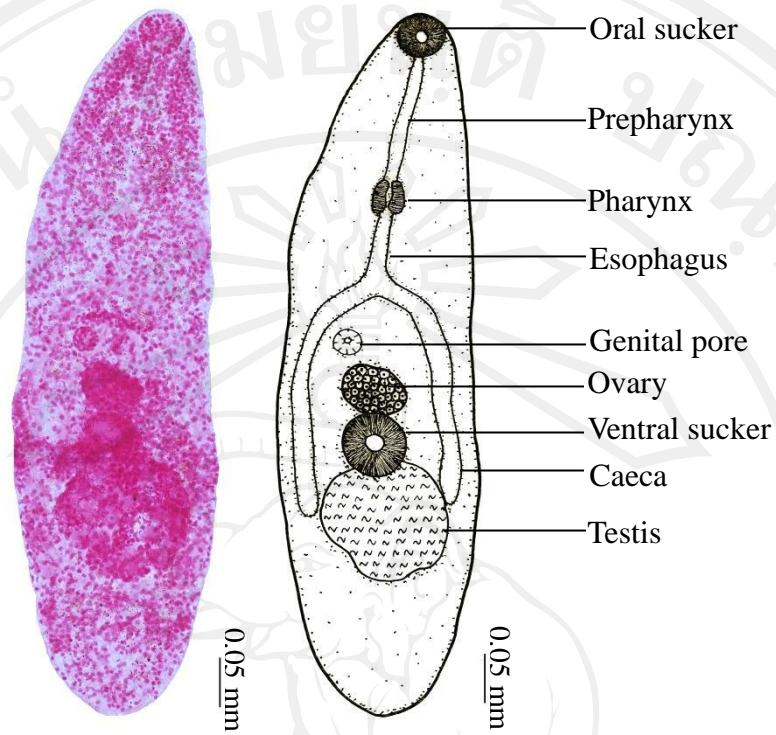
3. *Ganeo tigrinus*



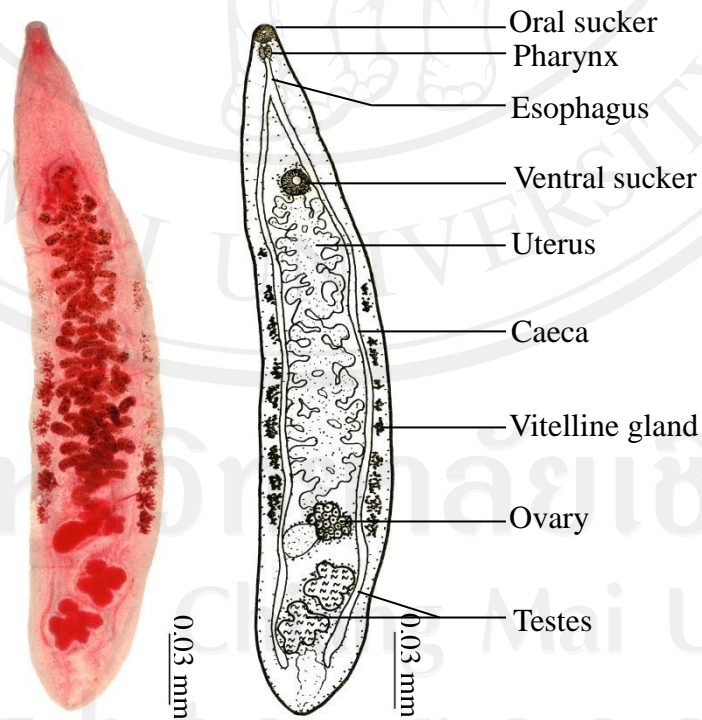
4. *Haplorchis taichui*



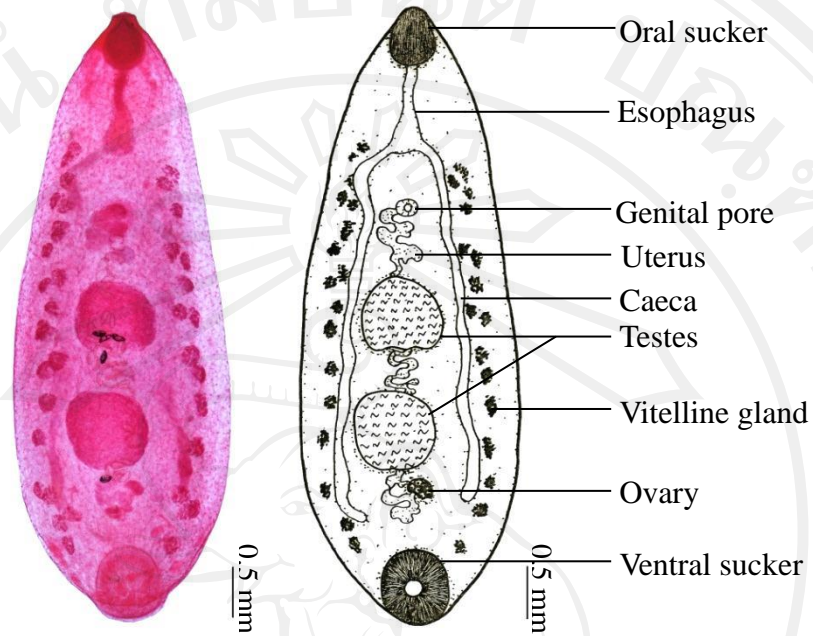
5. *Haplorchoides* sp.



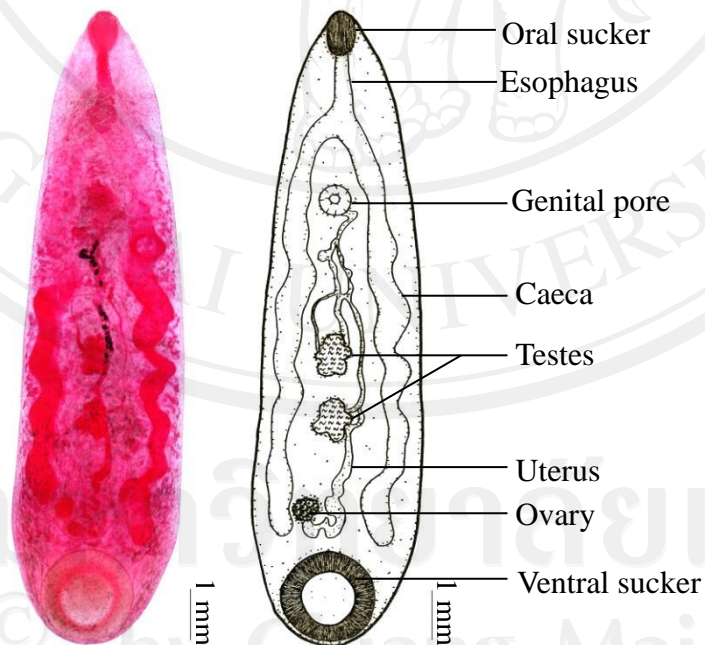
6. *Opisthorchis viverrini*



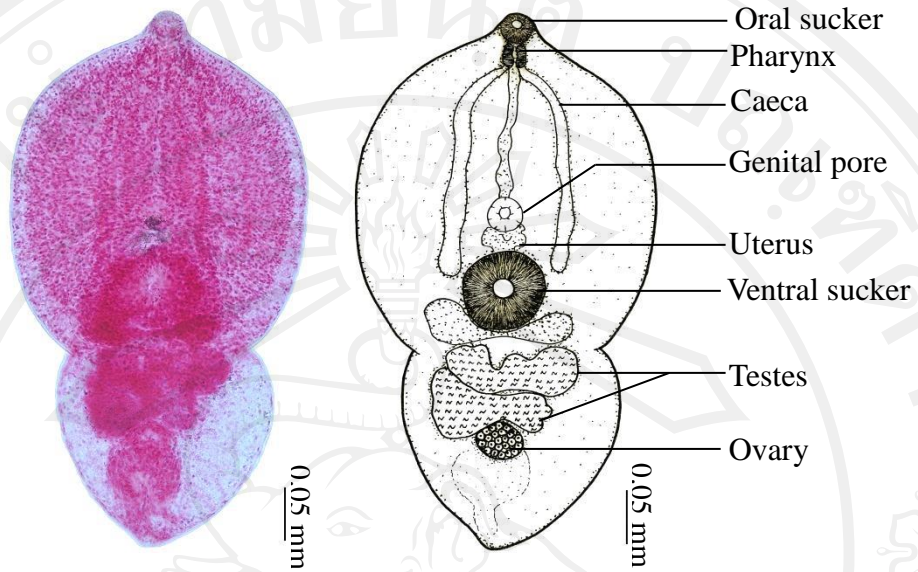
7. *Orthocoelium streptocoelium*



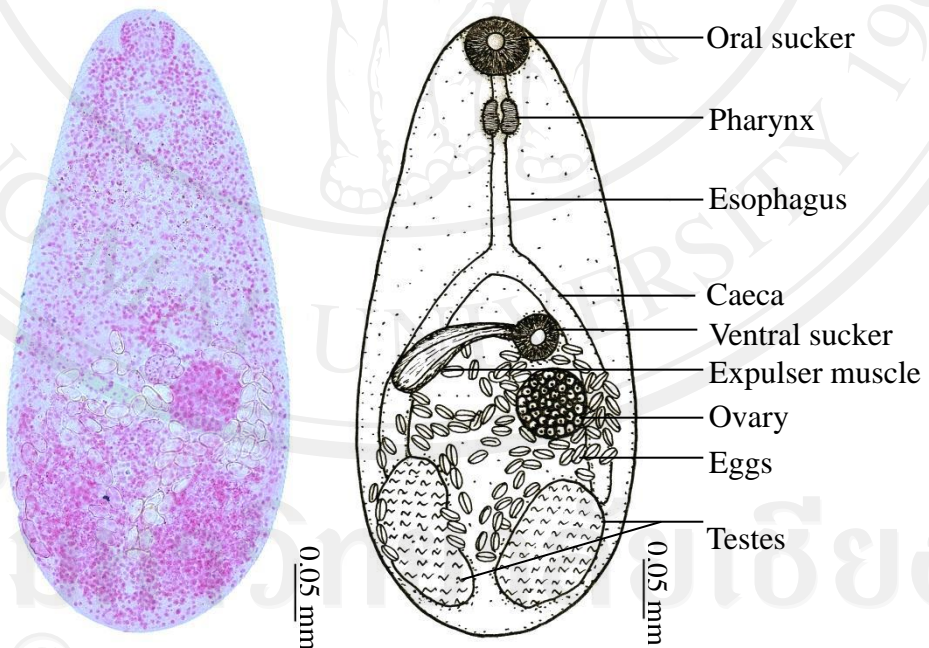
8. *Paramphistomum epicloctum*



9. *Posthodiplostomum* sp.



10. *Stellantchasmus* sp.



APPENDIX C

Sequences of ITS-2 region from this study

1. *Centrocestus caninus*

CCCCGGCAAGGATTGACCCAGCTCTTGTGTGATAAATGTGAAACTGCAAACCTGCTTTGA
ACATCGACATCTTGAACGCACATTGCGGCCATGGGTTTTCTGTGGCCACGCCTGTCCG
AGGGTCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTCTTGCCAGCC
GGCGTGATTTCTTGTGCTTTGCATGGGGTGCCGGATCTATGGCTTTTCCCTAATGTGC
CGGACGCAACCATCTCCAGGCTGGCGGTCTGGATGAGGAAGTGGCGGCGGAGTCGTGGC
TCAATGATACATATATATATATAAAGCGCGCTCTGTTGTCTATTCTTTGTGTGTGTT
CTCTGTGTTGTGTGTGGGGATGCATCCTATGCAAAAATTGCGCACTTTTCTAATGTGTG
TTATTTTCCCGACCTCTCATCACACGTAAATACACCCTCATTTTAAAAAATAAAT

2. *Fasciola gigantica*

GCGGATTAATATTGAGTGAGCATACTGTGTGATTAATGCAAACCTGCATACTGCTTTGAA
CATCGACATCTTGAACGCATATTGCGGCCATGGGTTAGCCTGTGGCCACGCCTGTCCGA
GGGTCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTG
GCGTGATCTCCTCTATGAGTAATCATGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATG
TATCCGGATGCACCCTTGTCTTGGCAGAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGT
GGTTTAATAATCGGGTTGGTACTCAGTTGTGAGTGTGTTCCGGCGATCCCCTAGTCGGCA
CACTCATGATTTCTGGGATAATTCCATACCAGGCACGTTCCGTTACTGTTACTTTGTCA
TTGGTTTTGATGCTGAACTTGGTCATGTGTCTGATGCTATTTTCATATAACGACGGTACCC
TTCGTGGTCTGTCTTCCCTGACCTCGGTTCCAGACGTGATTACCCGCTGAATTTAAGAATA
AA

3. *Fasciola gigantica* (Vietnam)

GGGGGCTTATGGAGAGCGCAGCCACTGTGTGATTAATGCAAACCTGCATACTGCTTTGAA
CATCGACATCTTGAACGCATATTGCGGCCATGGGTTAGCCTGTGGCCACGCCTGTCCGA
GGGTCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTG
GCGTGATCTCCTCTATGAGTAATCATGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATG
TATCCGGATGCACCCTTGTCTTGGCAGAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGT
GGTTTAATAATCGGGTTGGTACTCAGTTGTCAGTGTGTTCCGGCGATCCCCCAGTCGGCA
CACTCATGATTTCTGGGATAATTCCATAACCAGGCACGTTCCGTTACTGTTACTTTGTCA
TTGGTTTGATGCTGAACTTGGTCATGTGTCTGATGCTATTTTCATATAACGACGGTACCC
TTCGTGGTCTGTCTTCCCTGACCTCGGTTCCAGACGTGATTACCCGCTGAATTTAAGCATA
A

4. *Fischoederius elongatus*

GGGTTCGATGAGAGCGCAGCCACTGTGTGATTAATGTGAACTGCATACTGCTTTGAACA
TCGACATCTTGAACGCACATTGCGGCCACGGGTTTTCTGTGGCCACGCCTGTCCGAGG
GTCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGAATCTGCCAGCTGGC
GTGATTTCCCTCTGTGGTTCGCCACGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTC
TCCGGACACAACCTGCGTCTTGCTGGTAGCGCAGACGAGGGTGTGGCGGTAGAGTCGTGG
CTCAGTGAACGTAAATGGTAGCACGCTCTATTGTTGTGCCTTTGTTAGTGTAACCTGGTT
TGAGATGCTATTGCTGTTTCGTCCAATAATGATCACCCACTGTGGTGTCAATTACCTGA
CCTCGGATCAGACGTGAATACCCGCTGAATTTAAGCATAAA

5. *Haplorchis taichui*

CCCCCGGATAATAAGTAAAAGCTCTAGTTGTGATAAATGTGAACTGCCCAAAGCTTTGA
ACATCGACATCTTGAACGCACATTGCGGCCATGGGTTTTCTGTGGCCACGCCTGTCCG
AGGGTCGGCTTATAAACTATCACGACGCCCAAAATAGTCGTGGCTTGGGTTCTGCCAGCT
GGCGTGATTTCCCTTGTGCTTTTGCATAGGGTGCCAGATCTATGGCTTTTCCCTAATGTG
CCGGACGCAACCATGTCTTGGCTGACGGCCTGGATGAGGAAGTGGCGGCGGAGTCGTGG

CTCAATGAAAATTGTCCGCGCGCTCCAAAGCTTAACCTCTGTCTGGGCTGACGGCTTGG
ATGAGGAAGTGGCGGCGGAGTCGTGGCTCAATGAAAATTGTGCGCGCTCCAAAGCTTAA
CCTCTGTCTGGGCTGACGGTTTGGATGAGGAACCGGCGGCGGAGTCGTGGCTCAATGAA
AATTGTCCACGCG

6. *Haplorchoides* sp.

CGGCAATTAACCCCGCCACTTTTTGTGTGATTAATGTGAACTGCCTACTGCTTTGAACAT
CGACATCTTGAACGCATATTGCGGCCATGGGTTTTCTGTGGCCACGCCTGTCCGAGGG
TCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTCTTGCCAGCTGGCG
TGATTTCCCTGTGCTTTTTGTGTGGGGTGCCAGATCTATGGCTTTTCTTAATGTGCCGG
ACGCATCCACATTCGGGCTGTATACCGGGATGAGGAAGTGGCGGCAGAGTCGTGGCTCA
TTTTGTGAACTGTATAAATGCGCGCTCTGCTGTCTAACCTGTCTTGGTTGAAGCTTGAT
GTGTCAATGCATCTGATGCAAATTTTGGTGCCTTGCCTACTATTCTGACCTCGGATC
AGACGTGAATACCCGCTGAATTTAACCATAA

7. *Opisthorchis viverrini*

TCGAAGCTGAAAGGGGCGCTGCCTACTGTGTGATTAATGCGAACTGCATACTGCTTTGA
ACATCGACATCTTGAACGCATATTGCGGCCATGGGTTTGCCTGTGGCCACGCCTGTCCG
AGGGTCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTCTTGCCAGCT
GGCATGATTTCCCCGCGCAATTGTGTGGGGTGCCGGATCTATGGCTTTTCCCAATGTG
CCGGACGCAACCATGTCTGGGCTGACTGCCTAGATGAGGGGGTGGCGGCGGAGTCGTGG
CTCAATTGTTGTTATTGTTGTTGTGAATGCGCGCGCTCCGTTGTTGTTCTTTGTCTTT
GGTTGAGGCTCCAGTAGTGGCAATGCATTCGATGCAAATCGGTTTTGCACTTTGGTGCT
TAACAACCTTCTGACCTCGGATCAGACGTGATTACCCGCTGAATTTAAGAATAAAN

8. *Orthocoelium streptocoelium*

GGTTCTATAAGAGCGCAGCCACTGTGTGATTAATGTGAACTGCATACTGCTTTGAACAT
CGACATCTTGAACGCACATTCGGGCCACGGGTTTTCTGTGGCCACGCCTGTCCGAGGG

TCGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGAATCTGCCAGCTGGCG
TGATTTCCCTCTGTGGTTCCGCCACGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTCC
CCGGACACAACCGCGTCTTGCTGGTAACGCAGACGAGGGTGTGGCGGTAGAGTCGTGGC
TCAGTGAAGTGTAAATGGTAGCACGCTCTACTGTTGTGCCTTTGTTAGTGTAAGTGGTTT
GAGATGCTATTGCTGTCCGTCCAATCATGATCACCTACTGTGGTGTTCATTTACCTGAC
CTCGGATCAGACGTGAATACCCGCTGAATTTAAGCATAAA

9. *Paramphistomum epiclitum*

TAATGAGGAGCGCAGCCACTGTGTGATTAATGTGAACTGCATACTGCTTTGAACATCGA
CATCTTGAACGCACATTGCGGCCACGGGTTTTCCCTGTGGCCACGCCTGTCCGAGGGTCCG
GCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGAATCTGCCAGCTGGCGTGA
TTTTCCCTCTGTGGTTTGCCACGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTCTCCG
GACACAAGTGCCTTTGCTGGTAGCGCAGACGAGGGTGTGGCGGTAGAGTCGTGGCTCA
GTGAACTGTAAATGGTAGCACGCTCTATTGTTGTGCCTTTGTTAGTGCAGCTGGTTTGAG
ATGCTATTGCTGTCTGTCCAATGACGATCACCTACTGTGGTGTTCATTACCTGACCTC
GGATCAGACGTGAATACCCGCTGAATTTAAGCATA

10. *Stellantchasmus falcatus*

CGAGGGGCGGTTGGGGCGCAGCCACTGTGTGATTAATGTGAACTGCCTACTGCTTTGAA
CATCGACATCTTGAACGCACATTGCGGCCACGGGTTTTCCCGTGGCTACGCCTGTCCGA
GGGTCCGCTTATAAACTATCACGACGCCATAAAGTCGTGGCTTGGGTCTTGCCAGCTG
GCGTGATATCCCTGCGTCTGTTGTAGGGTGCCGGATCTGTGGCTTTTCCCAATGTGCC
GGATGCAACCATATCTAGGCAGGCTGCCTGGAAACGGGGGTGACGGCGGAGTCGTGGCT
CAATTGCTAATGTGAATAATGTGCGCGCTCCGTTGTCAATTATTTACCCGATGTTTGGC
TGATGCTTTGATATGGCAATGCACCTGACTATTTGTTTTGCACTGACGTGCCAACCCAT
CCTGACCTCGGATCAGACGTGAATACCCGCTGAATTTAAGCATAAAC

APPENDIX D

Sequences of ITS-2 region derived from GenBank

Fasciola gigantica 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GenBank: HQ700438.1

GCCCGACCCGCTAGGGATCACTGTGTGATTAATGCAAACCTGCATACTGCTTTGAACATC
GACATCTTGAACGCATATTGCGGCCATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGT
CGGCTTATAAACTATCACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGT
GATCTCCTCTATGAGTAATCATGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATC
CGGATGCACCCTTGTCTTGGCAGAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTT
TAATAATCGGGTTGGTACTCAGTTGTCAGTGTGTTCCGGCGATCCCCTAGTCGGCACACT
CATGATTTCTGGGATAATTCCATACCAGGCACGTTCCGTTACTGTTACTTTGTCATTGG
TTTGATGCTGAACTTGGTCATGTGTCTGATGCTATTTTCATATAACGACGGTACCCTTCG
TGGTCTGTCTTCTGACCTCGGTTCCAGACGTGATTACCCGCTGAATTTTAAGCATAA

Fasciola sp. Khanh.Ct.5.1 genes for 5.8S rRNA, ITS2, 28S rRNA

GenBank: AB536921.1

CAACTGTGTGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCAT
ATTGCGGCCATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTTCGGCTTATAAACTATC
ACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGT
AATCATGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTC
TTGGCAGAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTAATAATCGGGTTGGT
ACTCAGTTGTCAGTGTGTTCCGGCGATCCCYAGTCGGCACACTCATGATTTCTGGGATA
ATTCCATACCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTTGATGCTGAACTTG

GTCATGTGTCTGATGCTATTTTCATATAACGACGGTACCCTTCGTGGTCTGTCTTCCTGA
CCTCGGTTTCAGACGTGATTACCCGCTGAACTTAAGCATATCACTAA

***Fasciola gigantica* genes for 5.8S rRNA, ITS2, 28S rRNA, partial and complete
sequence, isolate: C-Bf 202**

GenBank: AB553695.1

CCAACCTGTGTGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCA
TATTGCGGCCATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTCGGCTTATAAACTAT
CACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAG
TAATCATGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGT
CTTGGCAGAAAGCCGTGGTGGTGCAGTGGCGGAATCGTGGTTAATAATCGGGTTGG
TACTCAGTTGTCAGTGTGTTTTGGCGATCCCCTAGTCGGCACACTCATGATTTCTGGGAT
AATTCATACCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCTGAACTT
GGTCATGTGTCTGATGCTATTTTCATATAACGACGGTACCCTTCGTGGTCTGTCTTCCTG
ACCTCGGTTTCAGACGTGATTACCCGCTGAACTTAAGCATATCACTAA

***Fasciola gigantica* genes for 18S rRNA, 5.8S rRNA and internal transcribed spacer
2, partial sequence**

GenBank: AB010977.1

TGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCGGC
CATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTCGGCTTATAAACTATCACGACGCC
CAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGTAATCATGT
GAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTCTTGGCAGA
AAGCCGTGGTGGTGCAGTGGCGGAATCGTGGTTAATAATCGGGTTGGTACTCAGTT
GTCAGTGTGTTCCGGCGATCCCCTAGTCGGCACACTCATGATTTCTGGGATAATTCCATA
CCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCTGAACTTGGTCATGTG
TCTGATGCTATTTTCATATAACGACGGTACCCTTCGTGGTCTGTCTTCCTGACCTCGGTT
CAGACGTGATTACCCGCTGAACTTAAGCATATC

***Fasciola* sp. genes for 18S rRNA, 5.8S rRNA and internal transcribed spacer 2, partial sequence**

TGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCGGC
CATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTTCGGCTTATAAACTATCACGACGCC
CAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGTAATCATGT
GAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTCTTGGCAGA
AAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTAATAATCGGGTTGGTACTCAGTT
GTCAGTGTGTTTCGGCGATCCCCAGTCGGCACACTCATGATTTCTGGGATAAATCCATA
CCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCTGAACTTGGTCATGTG
TCTGATGCTATTTTCATATAACGACGGTACCCTTCGTGGTCTGTCTTCCTGACCTCGGTT
CAGACGTGATTACCCGCTGAACTTAAGCATATC

***Fasciola gigantica* genes for 18S rRNA, 5.8S rRNA and internal transcribed spacer 2, partial sequence**

GenBank: AB010976.1

TGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCGGC
CATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTTCGGCTTATAAACTATCACGACGCC
CAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGTAATCATGT
GAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTCTTGGCAGA
AAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTAATAATCGGGTTGGTACTCAGTT
GTCAGTGTGTTTGGCGATCCCCTAGTCGGCACACTCATGATTTCTGGGATAAATCCATA
CCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCCGAACTTGGTCATGTG
TCTGATGCTATTTTCATATAACGACGGTACCCTTCGTGGTCTGTCTTCCTGACCTCGGTT
CAGACGTGATTACCCGCTGAACTTAAGCATATC

***Fasciola gigantica* isolate SRS2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence**

GenBank: JN828958.1

TACTCTTACACAAGCGATACACGTGTGACCGTCATGTCATGCGATAAAAATTTGCGGAC
GGCTATGCCTGGCTCATTGAGGTCACAGCATATCCGATCACTGATGGGGTGCCTACCTG

TATGATACTCCGATGGTATGCTTGCGTCTCTCGGGGCGCTTGTCCAAGCCAGGAGAACG
GGTTGTACTGCCATGATTGGTAGTGCTAGGCTTAAAGAGGAGATTTGGGCTACGGCCCT
GCTCCCGCCCTATGAACTGTTTCATTACTACAATTACACTGTTAAAGTGGTATTGAATG
GCTTGCCATTCTTTGCCATTGCCCTCGCATGCACCCGGTCCTTGTGGCTGGACTGCACG
TACGTCGCCC GGCGGTGCCTATCCCGGGTTGGACTGATAACCTGGTCTTTGACCATACG
TACAACTCTGAACGGTGGATCACTCGGCTCGTGTGTCGATGAAGAGCGCAGCCAACCTGT
GTGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCGG
CCATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTCGGCTTATAAACTATCACGACGC
CCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGTAATCATG
TGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTCTTGGCAG
AAAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTTAATAATCGGGTTGGTACTCAGT
TGTCAGTGTGTTTTGGCGATCCCCTAGTCGGCACACTCATGATTTCTGGGATAATTCCAT
ACCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCTGAACTTGGTCATGT
GTCTGATGCTATTTTCATATAACGACGGTACCCTTCGT

***Fasciola gigantica* partial ITS1, 5.8S rRNA gene and partial ITS2, isolate FgCAY1
GenBank: AM850108.1**

ACCTGAAAATCTACTCTTACACAAGCGATACACGTGTGACCGTCATGTCATGCGATAAA
AATTTGCGGACGGCTATGCCTGGCTCATTGAGGTCACAGCATATCCGATCACTGATGGG
GTGCCTACCTGTATGATACTCCGATGGTATGCTTGCGTCTCTCGGGGCGCTTGTCCAAG
CCAGGAGAACGGGTTGTACTGCCATGATTGGTAGTGCTAGGCTTAAAGAGGAGATTTGG
GCTACGGCCCTGCTCCCGCCCTATGAACTGTTTCATTACTACAATTACACTGTTAAAGT
GGTATTGAATGGCTTGCCATTCTTTGCCATTGCCCTCGCATGCACCCGGTCCTTGTGGC
TGGACTGCACGTACGTCGCCC GGCGGTGCCTATCCCGGGTTGGACTGATAACCTGGTCT
TTGACCATACGTACAACCTCTGAACGGTGGATCACTCGGCTCGTGTGTCGATGAAGAGCG
CAGCCAACCTGTGTGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAAC
GCATATTGCGGCCATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTCGGCTTATAAAC
TATCACGACGCCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTAT
GAGTAATCATGTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCT
TGTCTTGGCAGAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTTAATAATCGGGT
TGGTACTCAGTTGTCAGTGTGTTCCGGCGATCCCCTAGTCGGCACACTCATGATTTCTGG

GATAATTCATAACCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCTGAA
CTTGATCATGTGTCTGATGCTATTTTCATATAACGACGGATCCCTTCGTGGTCTGTCTTC
C

***Fasciola gigantica* gene for ITS2**

GenBank: AB207149.1

TGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCGGC
CATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTTCGGCTTATAAACTATCACGACGCC
CAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGTAATCATGT
GAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTCTTGGCAGA
AAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTAATAATCGGGTTGGTACTCAGTT
GTCAGTGTGTTCCGGCGATCCCCTAGTCGGCACACTCATGATTTCTGGGATAATTCCATA
CCAGGCACGTTCCGTTACTGTTACTTTGTCATTGGTTTGATGCTGAACTTGGTCATGTG
TCTGATGCTATTTTCATATAACGACGGTACCCTTCGTGGTCTGTCTTCCTGACCTCGGTT
CAGACGTGATTACCCGCTGAACTTAAGCATATC

***Fasciola hepatica* isolate FhC4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence**

CTACTCTCACACAAGCGATACACGTGTGACCGTCATGTCATGCGATAAAAATTTGCGGA
CGGCTATGCCTGGCTCATTGAGGTCACAGCATATCCGAACACTGATGGGGTGCCTACCT
GTATGATACTCCGATGGTATGCTTGCCTCTCTCGGGCGCTTGTCCAAGCCAGGAGAAC
GGGTTGTACTGCCACGATTGGTAGTGCTAGGCTTAAAGAGGAGATTTGGGCTACGGCCC
TGCTCCCGCCCTATGAACTGTTTCATTACTACATTTACACTGTTAAAGTGGTACTGAAT
GGCTTGCCATTCTTTGCCATTGCCCTCGCATGCACCCGGTCCCTTGTGGCTGGACTGCAC
GTACGTCGCCCCGGCGGTGCCTATCCCGGGTTGGACTGATAACCTGGTCTTTGACCATAC
GTACAACTCTGAACGGTGGATCACTCGGCTCGTGTGTCGATGAAGAGCGCAGCCAACTG
TGTGAATTAATGCAAACCTGCATACTGCTTTGAACATCGACATCTTGAACGCATATTGCG
GCCATGGGTTAGCCTGTGGCCACGCCTGTCCGAGGGTTCGGCTTATAAACTATCACGACG
CCCAAAAAGTCGTGGCTTGGGTTTTGCCAGCTGGCGTGATCTCCTCTATGAGTAATCAT
GTGAGGTGCCAGATCTATGGCGTTTTCCCTAATGTATCCGGATGCACCCTTGTCTTGGCA

GAAAGCCGTGGTGAGGTGCAGTGGCGGAATCGTGGTTTAATAATCGGGTTGGTACTCAG
TTGTCAGTGTGTTTGGCGATCCCCTAGTCGGCACACTTATGATTTCTGGGATAAATTCCA
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TGTCTGATGCTATTTTCATATAGCGACGGTACCCTTCGTGGTCTGTCTCTAC

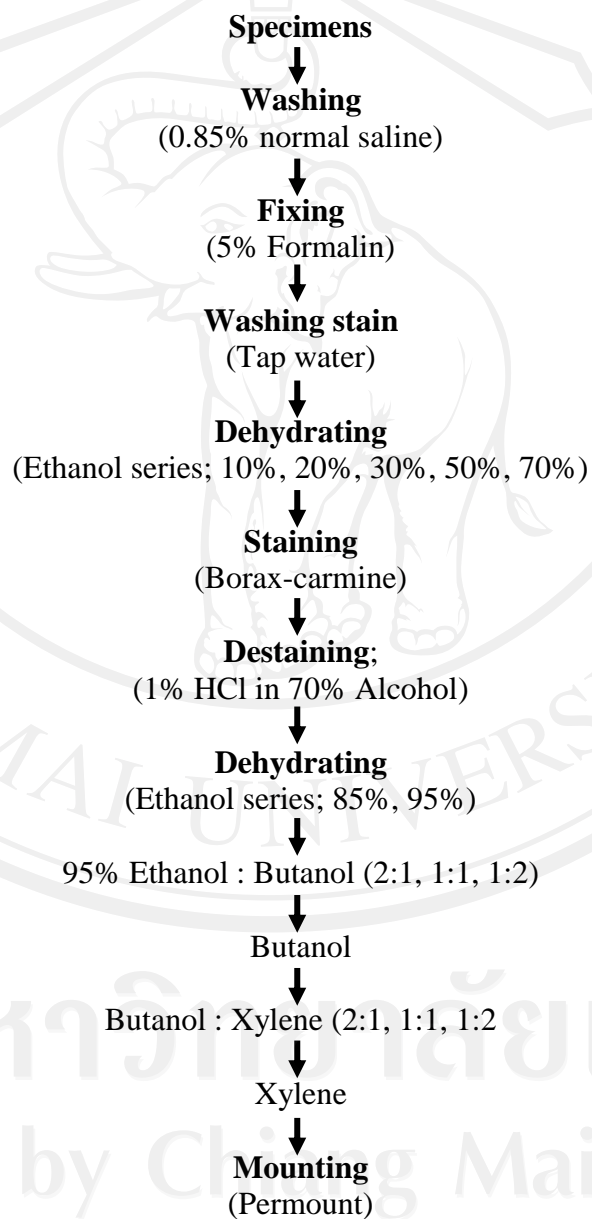
***Fasciola hepatica* isolate FhAM1 internal transcribed spacer 1, partial sequence;
5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2,
partial sequence**

ACCTGAAAATCTACTCTCACACAAGCGATACACGTGTGACCGTCATGTCATGCGATAAA
AATTTGCGGACGGCTATGCCTGGCTCATTGAGGTCACAGCATATCCGAACACTGATGGG
GTGCCTACCTGTATGATACTCCGATGGTATGCTTGCCTCTCTCGGGGCGCTTGTCCAAG
CCAGGAGAACGGGTGTACTGCCACGATTGGTAGTGCTAGGCTTAAAGAGGAGATTTGG
GCTACGGCCCTGCTCCCGCCCTATGAACTGTTTCATTACTACATTTACACTGTTAAAGT
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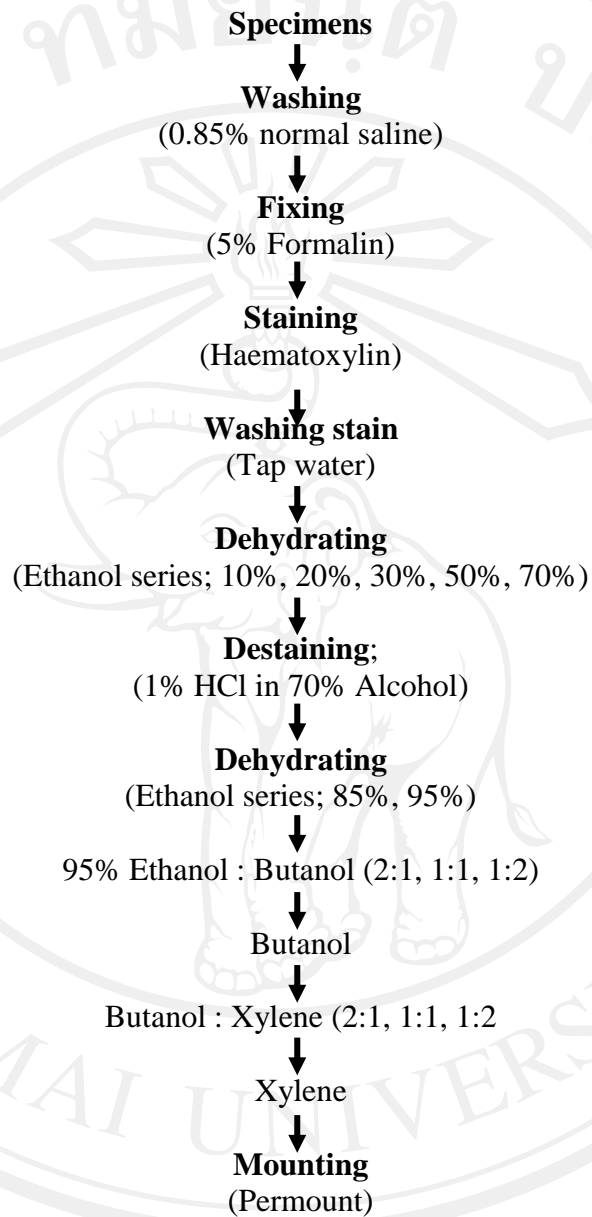
APPENDIX E

Processes of specimen preparation for permanent slide

1. Borax-carmin staining



2. Haematoxylin staining



APPENDIX F

Reagents and Stains preparation

1. Acid pepsin solution 1%

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

2. Borax carmine (Grenacher's alcoholic)

Borax carmine	8	g
70% ethanol	200	ml

Boil for ½ hour, cool and make up to 200 ml

3. Delifield alum haematoxylin

Aluminiun alum	3	g
Haematoxylin	0.6	g
Glycerine	15	ml
Methanol	15	ml
95% ethanol	4	ml
Distilled water	70	ml

Dissolve haematoxylin in 95% ethanol (1)

Dissolve Aluminium alum in distilled water (2)

Solution (1) + (2) add Glycerine and methanol. Stand in a sunny place to ripen for several weeks

4. Formalin 5%

40% Formadehyde	5	ml
Distilled water	95	ml

5. Normal saline 0.85%

Sodium chloride	8.5	g
Distilled water	1000	ml

APPENDIX G

Reagents preparation for molecular biological study

1. Agarose gel 1.4%

Agarose gel	1.4 g
1x Tris Boric acid EDTA buffer	100 ml

Dissolve 1.4 g agarose gel to 100 ml 1x Tris Boric acid EDTA buffer and boiling by using microwave oven and pour the gel when the agarose has cooled to about 55 °C on gel electrophoresis tray.

2. EDTA 0.5 M (pH 8.0)

EDTA.Na ₂ .2H ₂ O	186.12	g
NaOH pallet/10N NaOH solution		
Deionized water	1000	ml

Dissolve EDTA.Na₂.2H₂O 186.12 g in deionized water 800 ml, set pH 8.0 by add ~20 g NAOH while stirring vigorously, adjust the volume to 1000 ml and autoclave

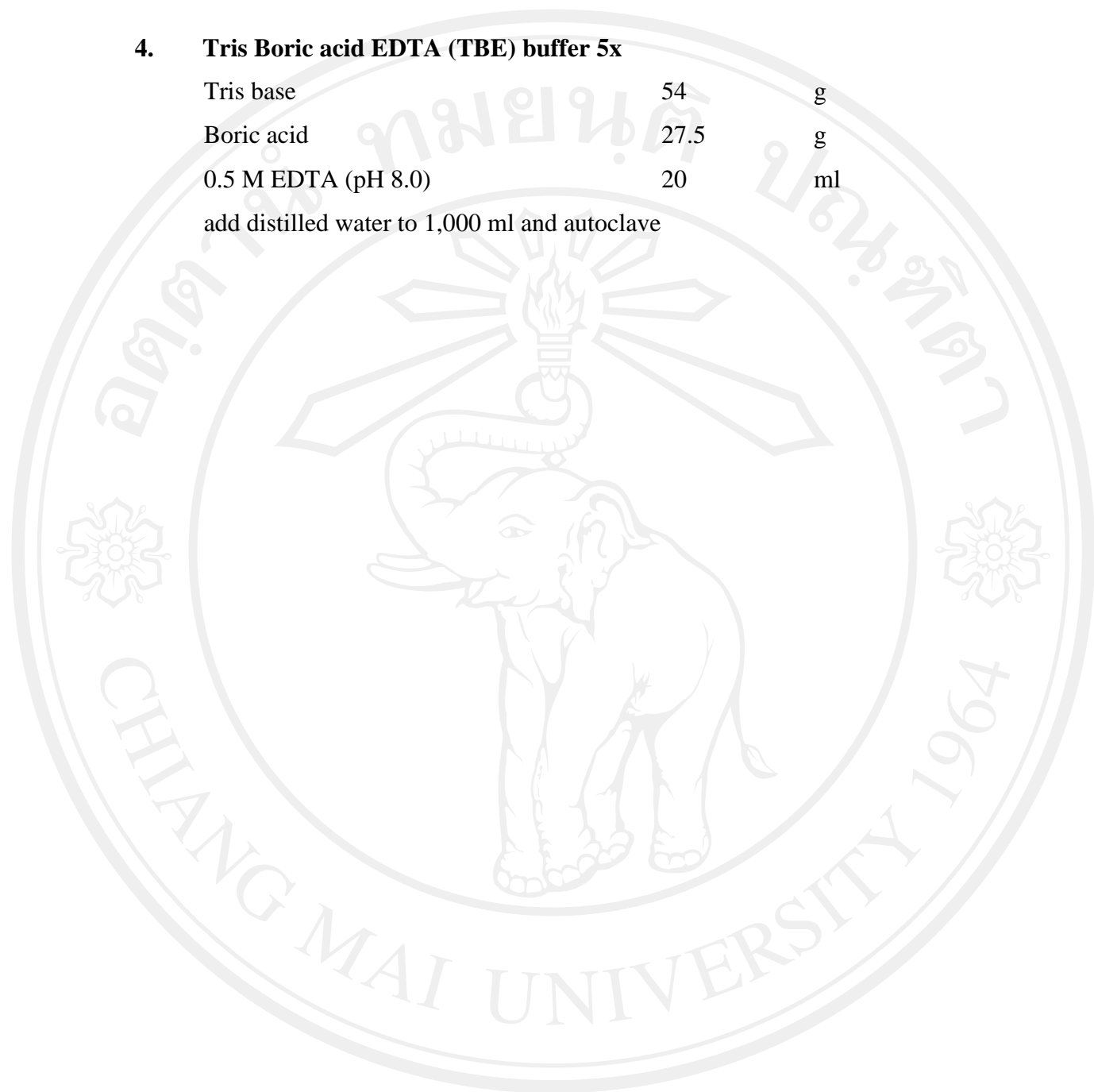
3. Ethidium bromide (10mg/ml stock solution)

Ethidium bromide	0.2	g
Distilled water	20	ml

Dissolve 0.2 g ethidium bromide to 20 mL water. Mix well and store at 4 °C in the dark.

4. Tris Boric acid EDTA (TBE) buffer 5x

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		



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