



RAPD designed for species-specific primer for *Bithynia siamensis goniomphalos*

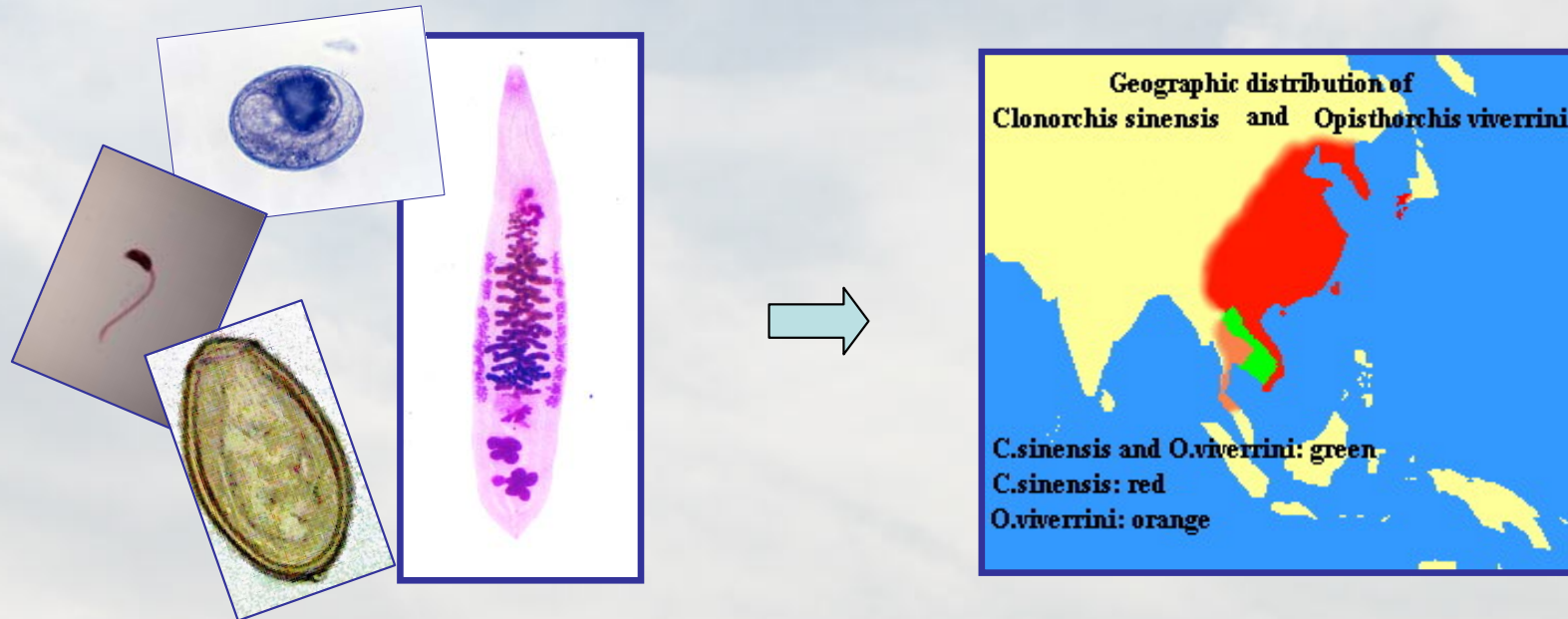
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Rationale and Background

- *Opisthorchis viverrini* is a human liver fluke endemic in Thailand, Lao PDR, Cambodia and south of Vietnam (WHO, 1995; Le et al., 2006).



- *O. viverrini* infection was a major public health problem in the Northeast, Thailand (Jongsuksuntigul, 2002)

In 2003, Sripa *et al.* reported that *O. viverrini* caused of biliary diseases, including

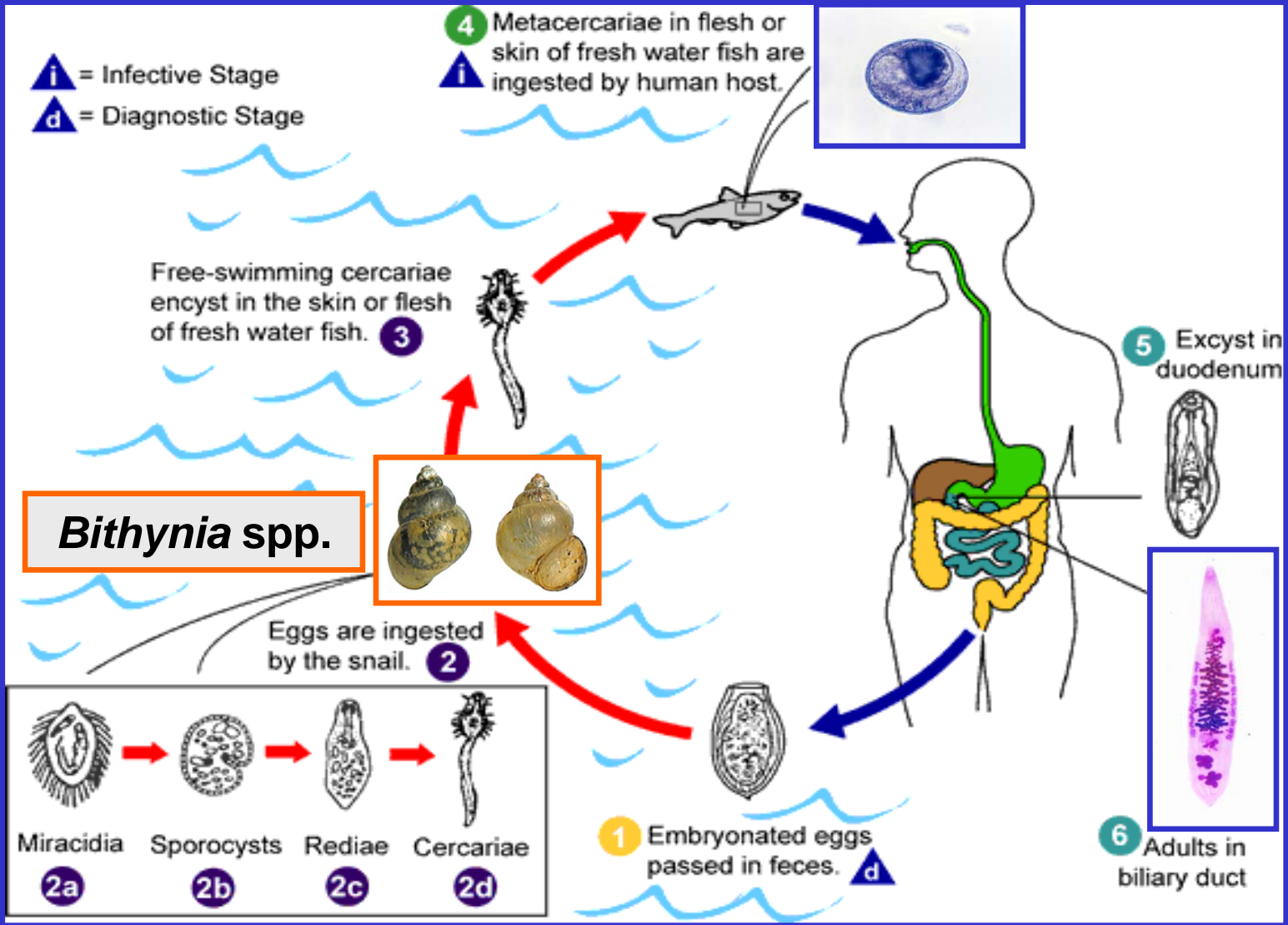
- Cholangitis**
- Obstructive jaundice**
- Hepatomegaly**
- Cholecystitis**
- Cholelithiasis**
- Cholangiocarcinoma (CCA)**

The life-cycle of *O. viverrini* requires 2 intermediate hosts

1st intermediate host ⇒ *Bithynia* spp.

2nd intermediate host ⇒ Cyprinoid fish

Life cycle of *O. viverrini*



Classification of *Bithynia* spp.

Phylum Molluska

Class Gastropoda

Subclass Prosobranchia

Order Mesogastropoda

Superfamily Rissooidea

Family Bithyniidae



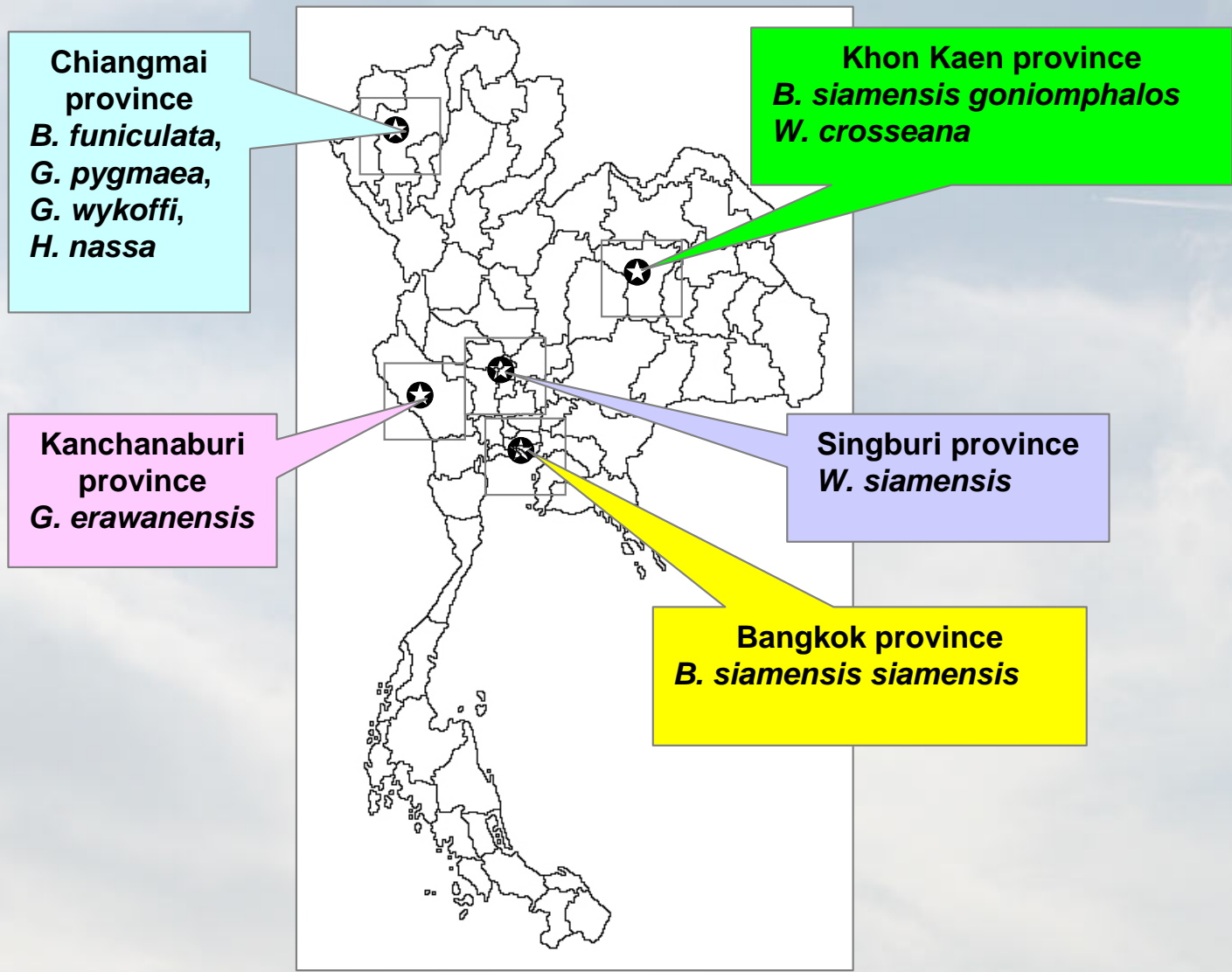
Snail samples



(Brandt, 1974)

Figure 2 The shell morphology of bithyniid snails;

A: *B. funiculata*, **B:** *B. siamensis goniomphalos*, **C:** *H. nassa*,
D: *B. siamensis siamensis*, **E:** *W. crosseana*, **F:** *W. siamensis*,
G: *G. wykoffi*, **H:** *G. pygmaea*, **I:** *G. erawanensis*



Thailand map shows the locations for bithyniid snail collection

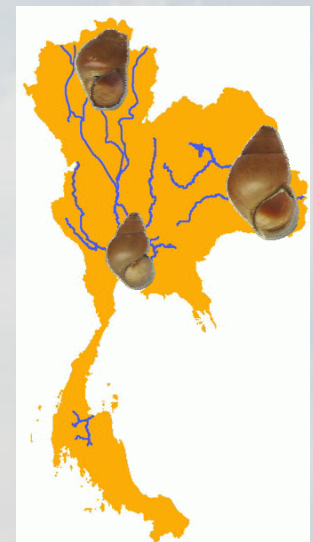


- Size
- Shape
- Color of shell,

Not easily identified

The appearance of these features may not be available due to the induction of the environment.

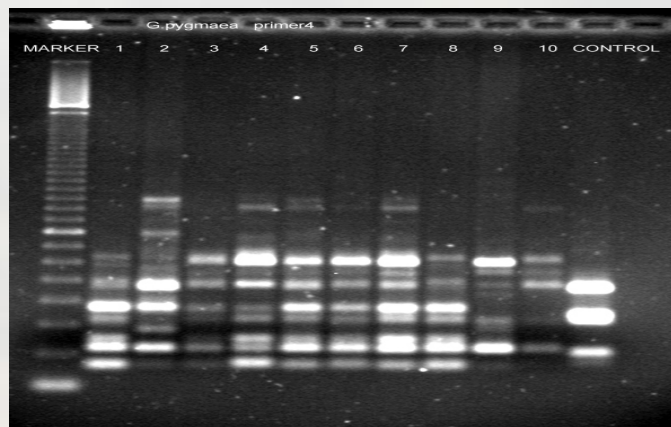
- Sympatric species
- Hybrid progeny



Molecular technique

Random Amplified Polymorphic DNA polymerase chain reaction (RAPD-PCR)

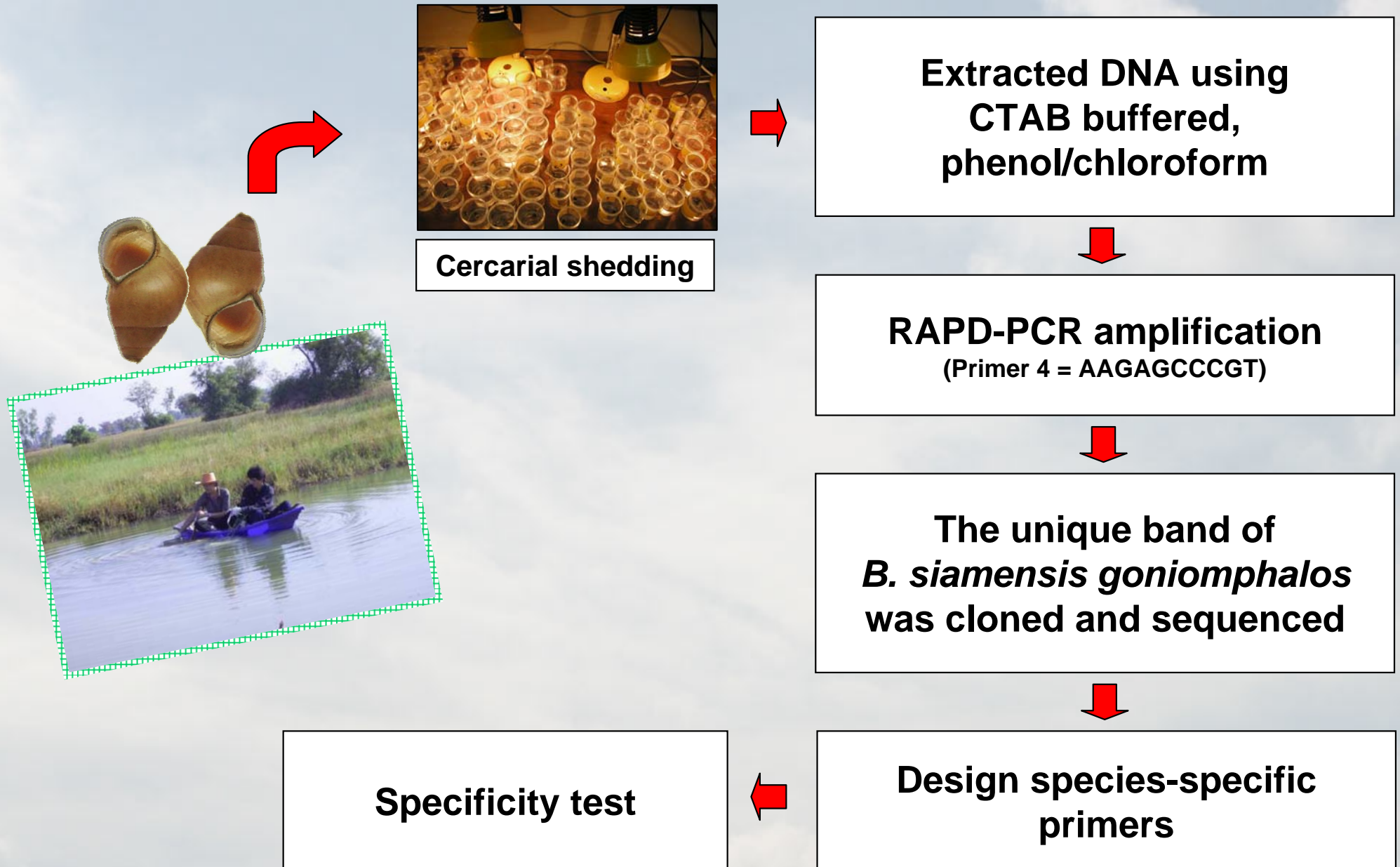
- Short random primer are used to amplify DNA. (about 10 base pair)
- Annealing at low stringency
- Single RAPD primer may detect several loci simultaneously

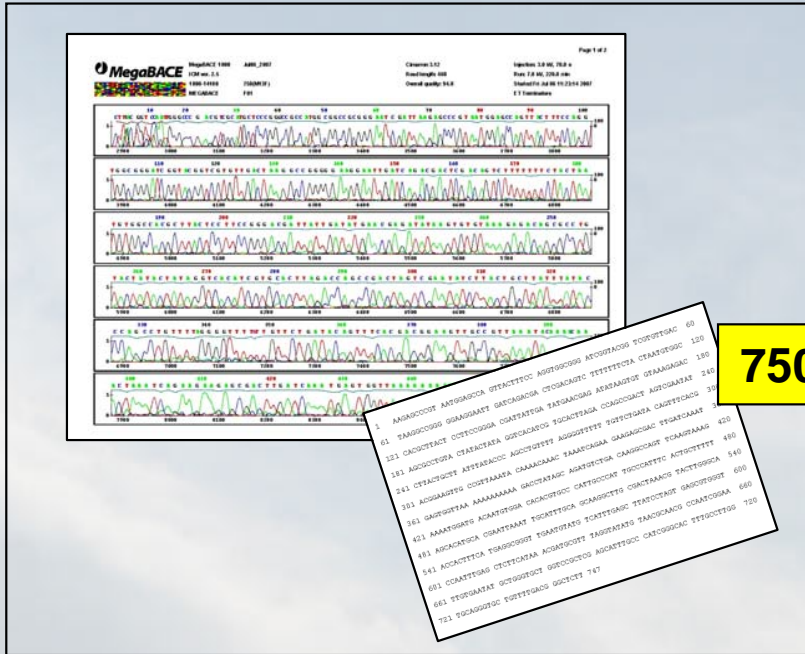


OBJECTIVE

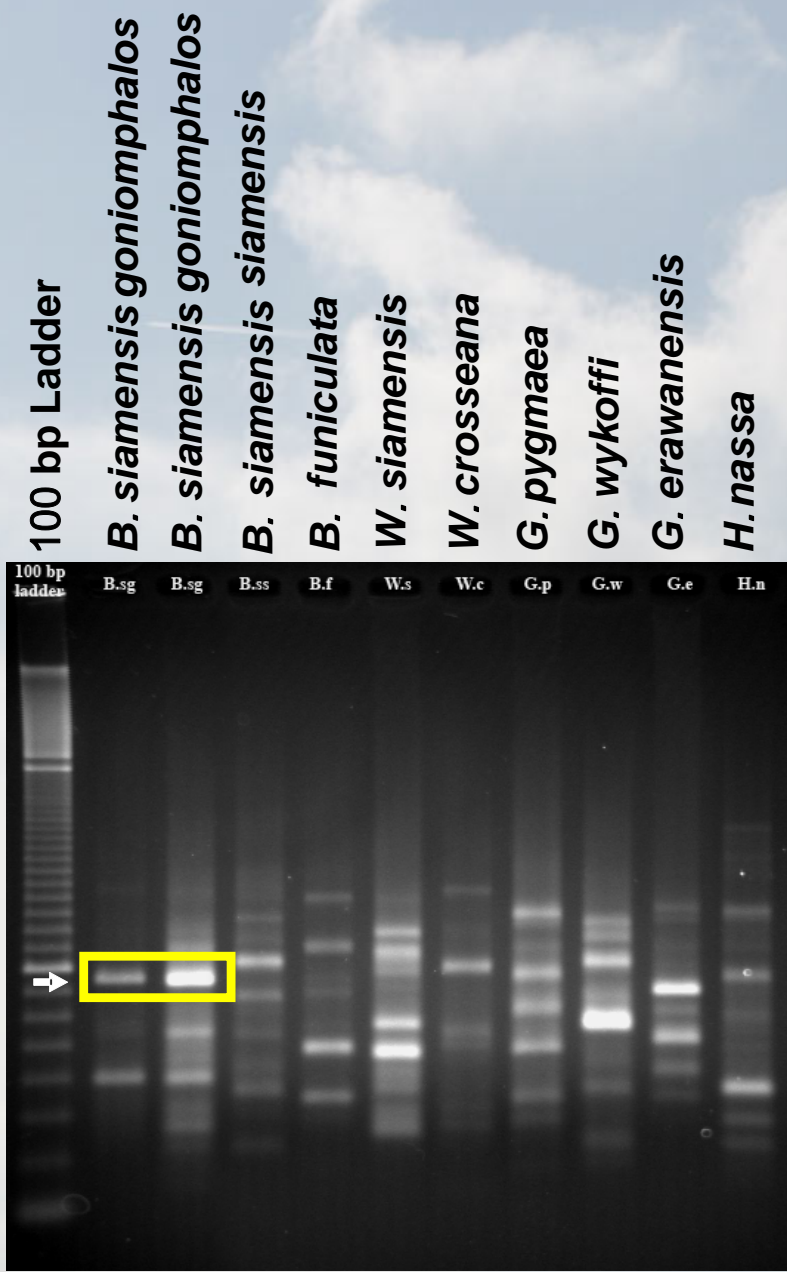
- ◆ Identification of *Bithynia siamensis goniomphalos* using species-specific primers by PCR technique

Schematic study design





750 bp



DNA sequencing at the laboratory of Biochemistry department, Faculty of Medicine, Khon Kaen University

DNA sequencing was designed species-specific primers by

Oligo 4.1 software

BSGF primer

```
1  AAGAGCCCGT AATGGAGCCA GTTACTTTCC AGGTGGCGGG ATCGGTACGG TCGTGTTGAC 60
61  TAAGGCCGGG GGAAGGAATT GATCAGACGA CTCGACAGTC TTTTTTCTA CTAATGTGGC 120
121 CACGCTTACT CTTCCGGGA CGATTATTGA TATGACGAG ATATAAGTGT GTAAAGAGAC 180
181 AGCGCCTGTA CTATACTATA GGTACATCG TGCACCTAGA CCAGCCGACT AGTCGAATAT 240
241 CTTACTGCTT ATTTATACCC AGCCTGTTTT AGGGGTTTTT TGTTCTGATA CAGTTTCACG 300
301 ACGGAAGTTG CCGTTAAATA CAAAACAAAC TAAATCAGAA GAAGAGCGAC TTGATCAAAT 360
361 GAGTGGTTAA AAAAAAAAAA GACCTATAGC AGATGTCTGA CAAGGCCAGT TCAAGTAAAG 420
421 AAAATGGATG ACAATGTGGA CACACGTGCC CATTGCCCAT TGCCCATTTT ACTGCTTTTT 480
481 AGCACATGCA CGAATTAAAT TGCATTTGCA GCAAGGCTTG CACTAAACG TACTTGGGCA 540
541 ACCACTTTCA TGAGGCGGGT TGAATGTATG TCATTTGAGC TTATCCTAGT GAGCGTGGGT 600
601 CCAATTTGAG CTCTTCATAA ACGATGCGTT TAGGTATATG TAACGCAACG CCAATCGGAA 660
661 TTGTGAATAT GCTGGGTGCT GGTCCGCTCG AGCATTTGCC CATCGGGCAC TTTGCCTTGG 720
721 TGCAGGGTGC TGTTTTGACG GGCTCTT 747
```

BSGR primer

Accession No. EU200236

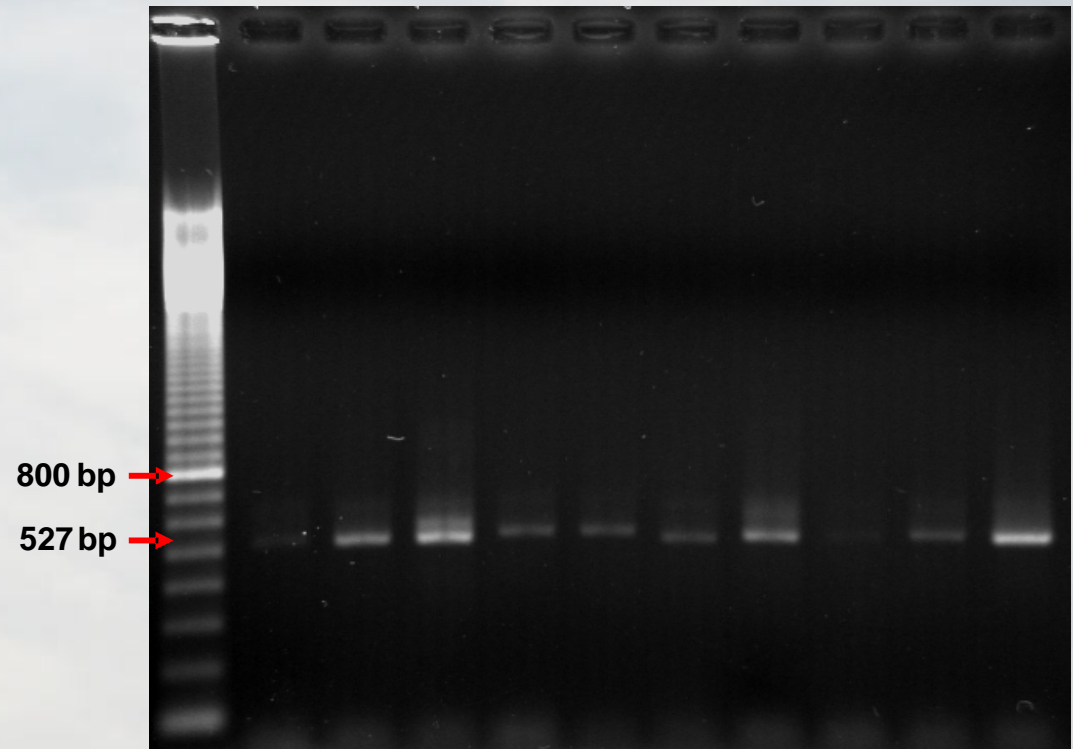
Results

Specificity of species-specific primer

1. Ten strains of *B. siamensis goniomphalos*



100 bp Ladder
Ubon Ratchathani
Maha Sarakham
BuriRum
Nakhon Phanom
Chaiyaphum
Nakhon Ratchasima
Loei
Sakon Nakhon
Si Sa Ket
Roi Et

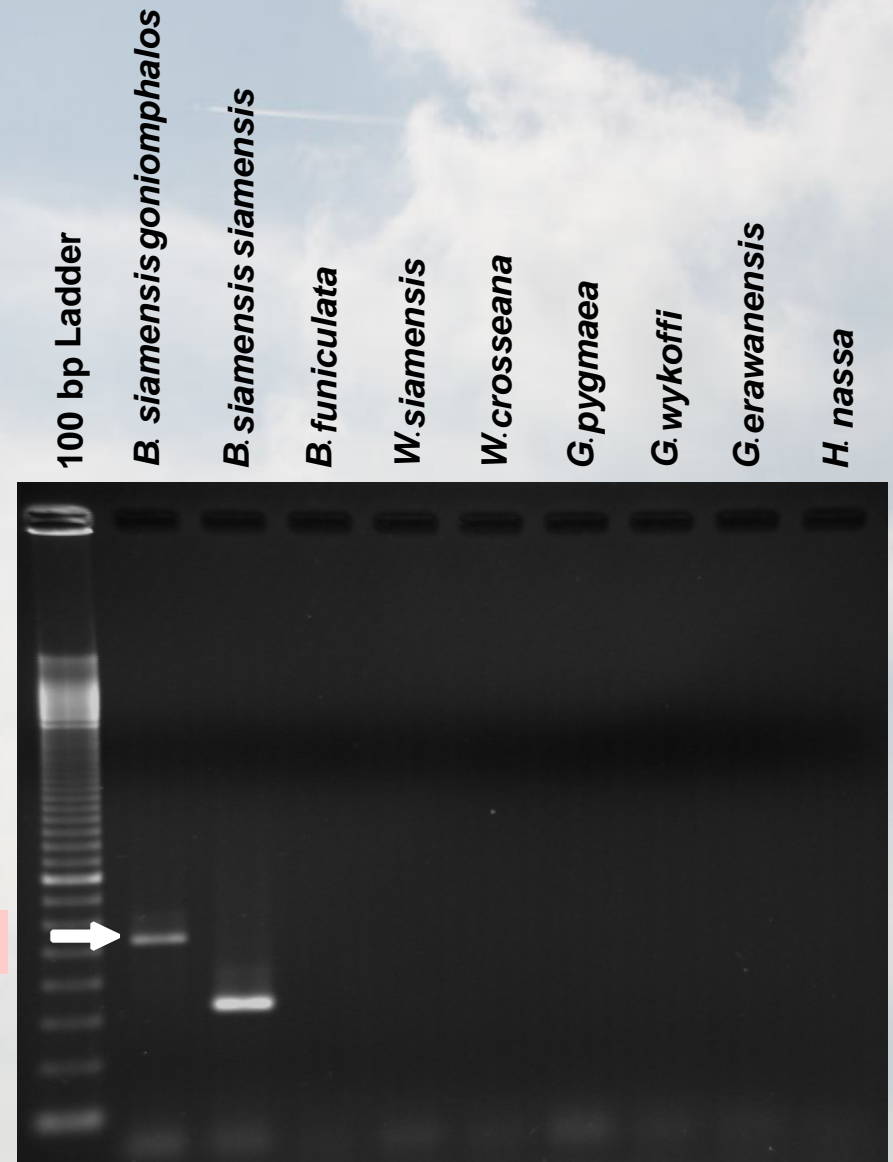


2. The other members in family Bithyniidae

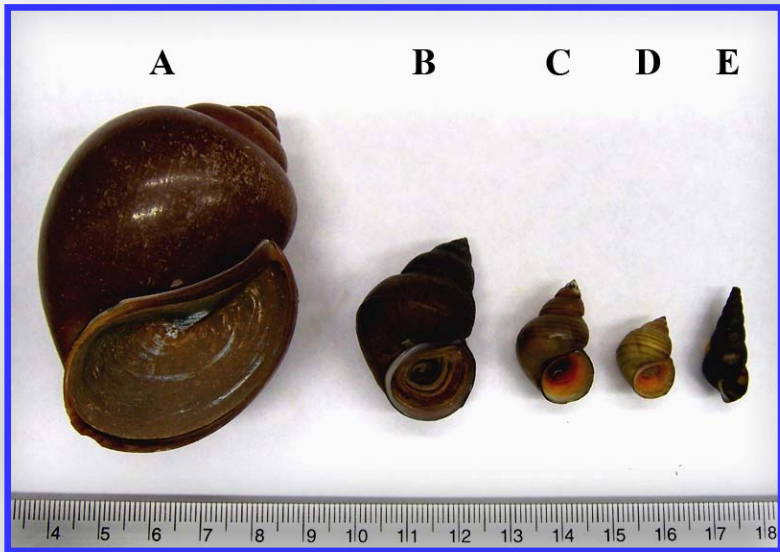


- A: *B. funiculata*, B: *B. siamensis goniomphalos*,
C: *H. nassa*, D: *B. siamensis siamensis*,
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G: *G. wykoffi*, H: *G. pygmaea*,
I: *G. erawannensis*

527 bp

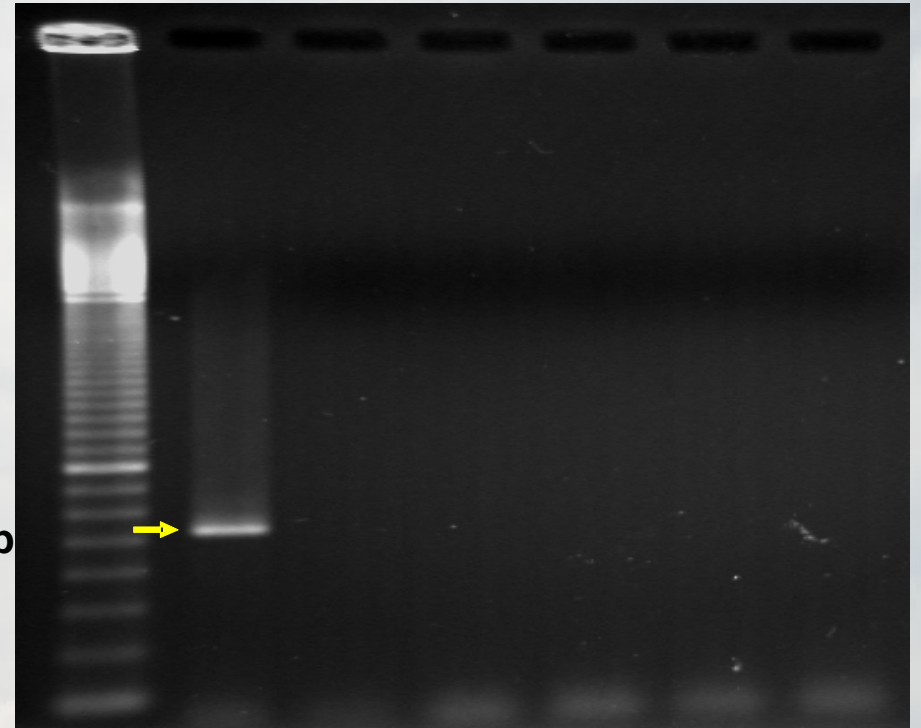


3. The snails out of family Bithyniidae



- A: *Pila polita*,
- B: *Filopaludina (S) martensi martensi*,
- C: *Filopaludina (F) sumatrensis speciosa*,
- D: *Filopaludina (F) sumatrensis polygramma*,
- E: *Adamietta housei*

527 bp



100 bp Ladder

B. siamensis goniomphalos

F. (F) sumatrensis speciosa

P. polita

F. (F) sumatrensis polygramma

F. (S) martensi martensi

Adamietta housei

CONCLUSION

RAPD-PCR could be applied for species differentiation which the unique band was investigated nucleotide sequences for species-specific primers synthesis.

The unique band from RAPD-PCR profiles was cloned and sequenced for designing species-specific primers to *B. siamensis goniomphalos*.



ACKNOWLEDGEMENTS



Dr. Smarn Tesana
Advisor



Dr. Thidarat Boonmars
Co-advisor



Dr. Paiboon Sithithaworn
Co-advisor



- ✦ **Dr. Duangduen Krailas**
- ✦ **Miss Radchadawan Ngern-klun**
for the snail samples supply in this study