

Molecular analysis of *Fasciola* flukes from Thailand based on the nuclear ITS1 region and mitochondrial DNA markers

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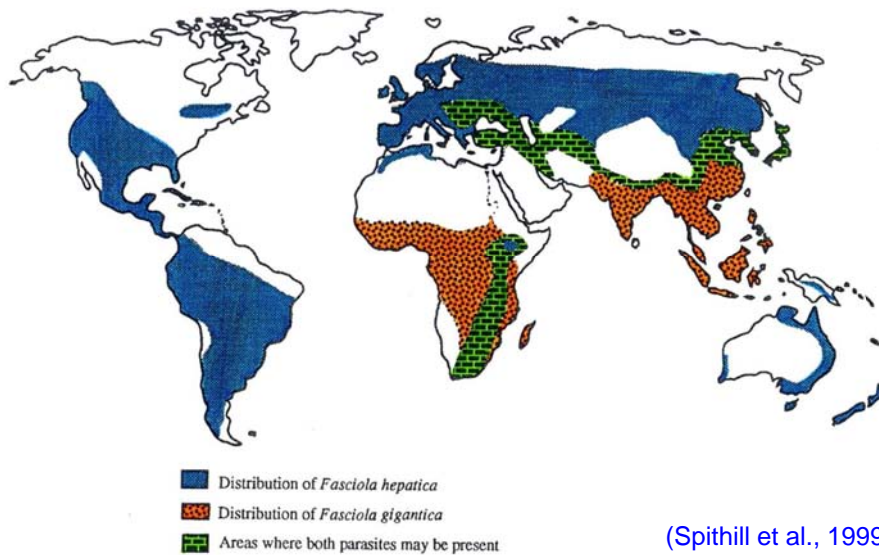
Fasciola spp.

- commonly known as liver flukes or flatworm
- caused of fascioliasis (zoonotic diseases: animal → human)
- hosts : domestic animals (cattle, goat, sheep etc.)
- intermediate host : lymanea snails (freshwater snail)
- symptoms : animal illness and potential loss of lives that causes of the economic losses in livestock.



Fasciola spp.

- Class : Trematode
- Genus *Fasciola* spp. can be divided into two main groups
- Distribution of *Fasciola* spp.
 - Temperate zone : *F. hepatica*
 - Tropical zone : *F. gigantica*



F. gigantica

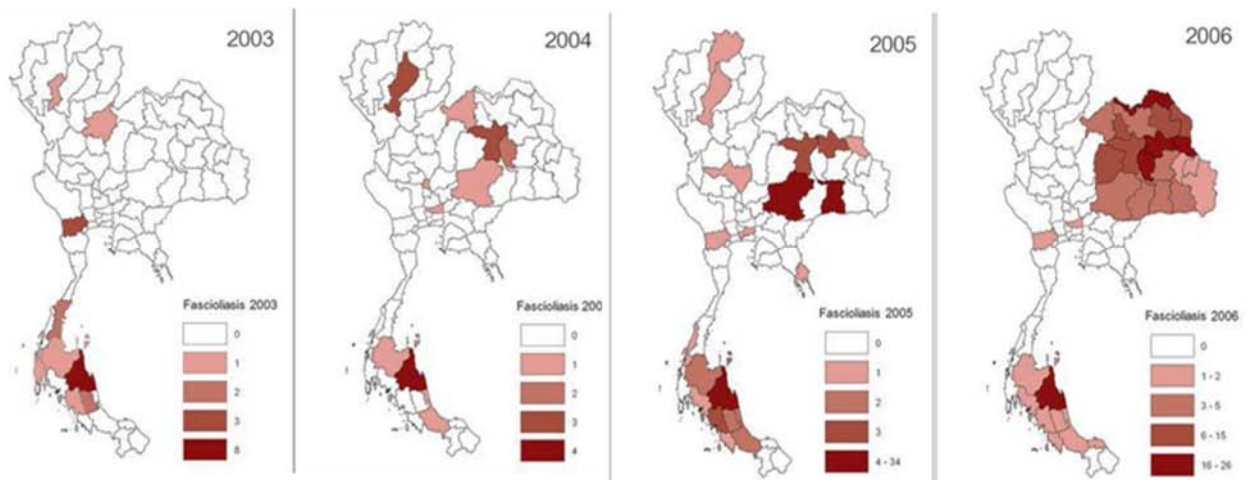


F. hepatica



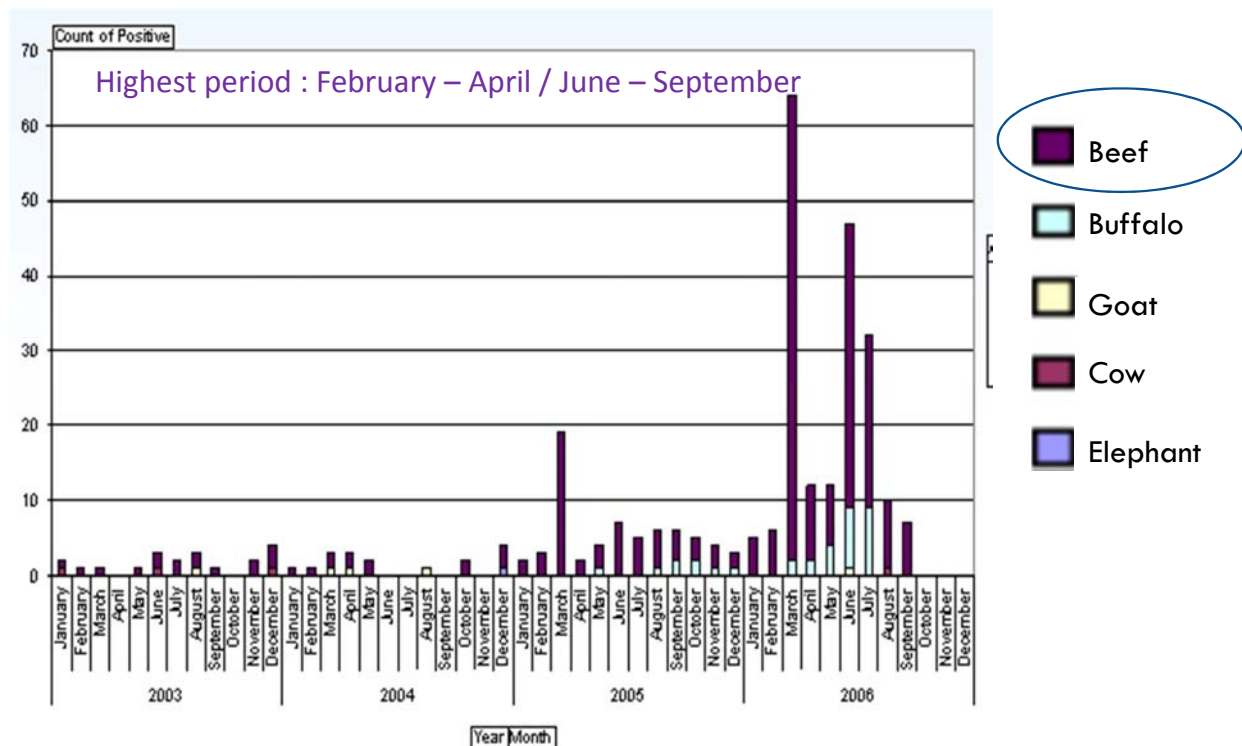
(Spithill et al., 1999)

Epidemiology of fascioliasis in livestock in each region of Thailand : 2003-2006 (National Institute of Animal Health)



* Fascioliasis were found more than other internal parasite samples.

Epidemiology of fascioliasis in livestock in each region of Thailand : 2003-2006 (National Institute of Animal Health)

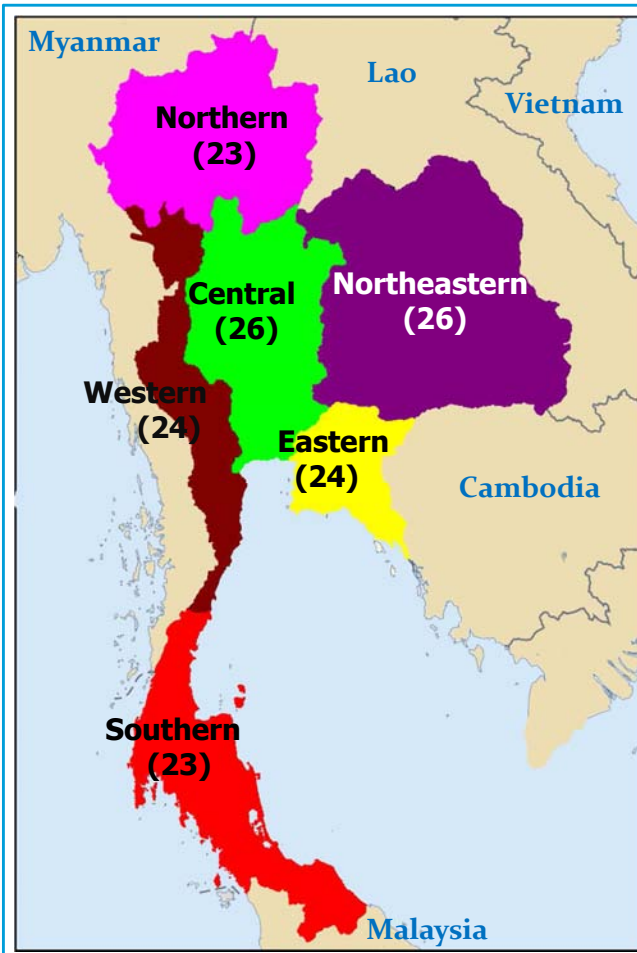


- **Research problem :**

Identification of haplotypes and phylogeny of *Fasciola* spp. in Thailand with those in neighboring countries are still not clear.

- **Objectives:**

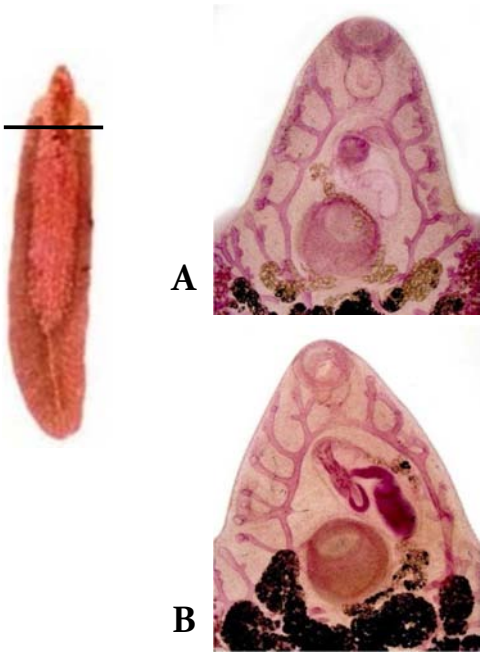
To characterize haplotypes of *Fasciola* samples obtained from different geographical regions of Thailand, and analyze phylogenetic relationship of the parasite haplotypes in Thailand and those in neighboring countries.



- 146 *Fasciola* samples were collected from cattle
- fixed in 70% ethanol



Analysis of spermic and aspermic *Fasciola* samples



Aspermic type

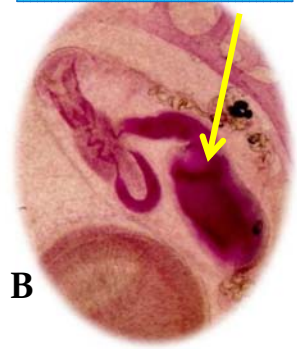
18 samples



Seminal vesicle

Spermic type

128 samples

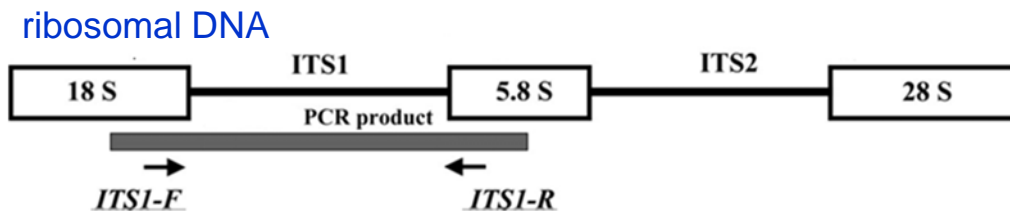


Fasciola samples stained with hematoxylin-carmin

Analysis of genotypes using ITS1

ITS (Internal transcribed spacer regions of ribosomal genomic DNA)

- sequences are believed to evolve without functional constraints
- ITS1 and ITS2 are accepted as a method for species identification



Analysis of genotypes using ITS1

1. Genomic DNA extraction
2. Amplification of ITS1 from 18S and 5.8S rDna by using primers :(Itagaki et al.,2005)

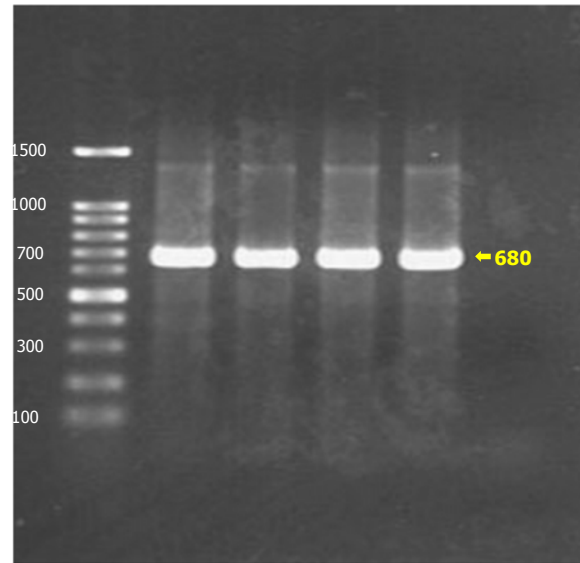
F_ITS1 : 5' TTGCGCTGATTACGTCCCTG 3'
R_ITS1 : 5' TTGGCTGCGCTCTTCATCGAC3'

3. Determine restriction fragment length polymorphisms (RFLP) by cutting with restriction enzyme RsaI

5'...GT AC...3'
3'...CA TG...5' Restriction site
(Ichikawa and Itagaki, 2010)

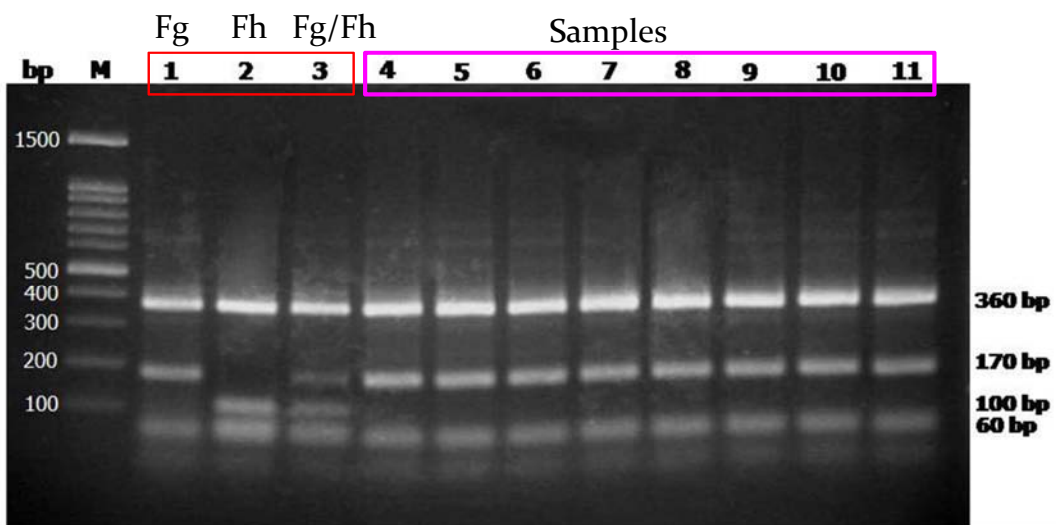


Polymerase chain reaction amplified ITS1 ribosomal DNA



PCR- ITS1

Polymerase chain reaction amplified ITS1 digested with restriction enzymes *Rsa*I



RFLP using *Rsa* I

Conclusion: All of the 146 specimens were *F. gigantea* type

Spermatogenesis and ITS1 genotyping of *Fasciola* samples in Thailand

Spermatogenesis	ITS1	Number of samples	Species identification
Spermic	Fg types	128	<i>F. gigantica</i>
Aspermic	Fg types	18	Parthenogenetic <i>Fasciola</i> sp.

Phylogenetic analysis by comparing partial sequence of ND1

- Mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit (ND1) used as a DNA barcode

1. Sequencing (535 bp)

Second set of primers for sequencing :

F_Ichi1 : 5' AGGTGTTGGGTTATATGCA 3'

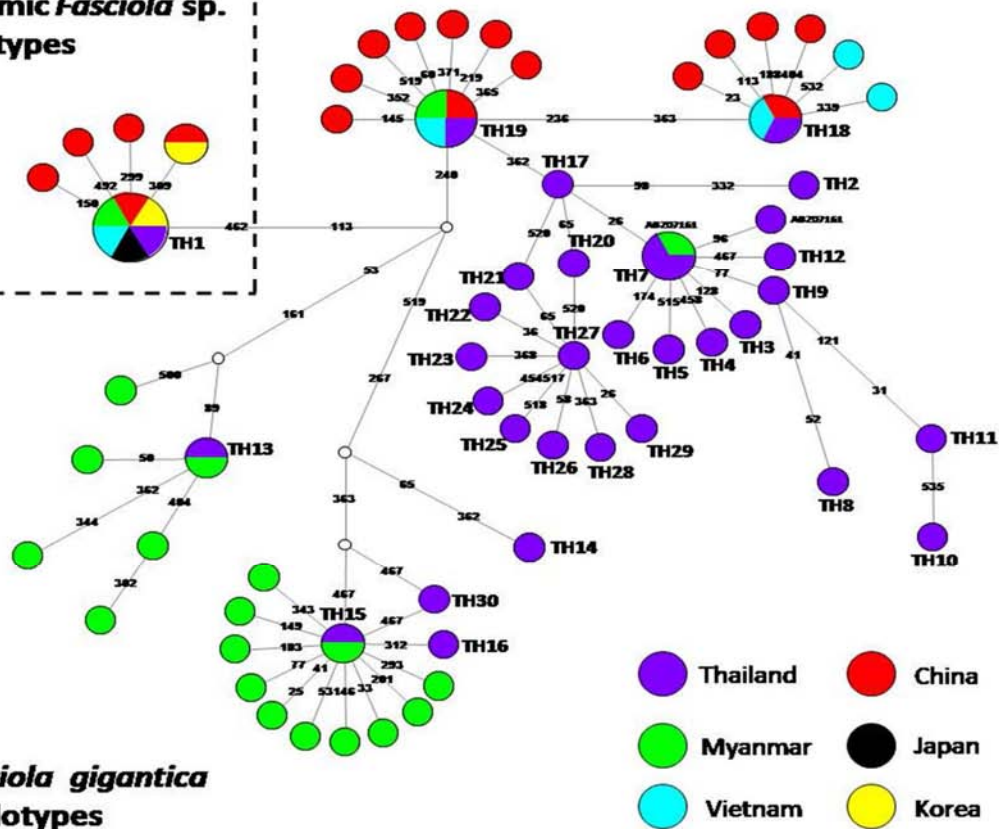
R_Ita2 : 5' GGAGTACGGTTACATTCACA 3'

3. Median-joining network analysis

ND1 haplotypes of *F. gigantica* and pathenogenetic *Fasciola* sp.

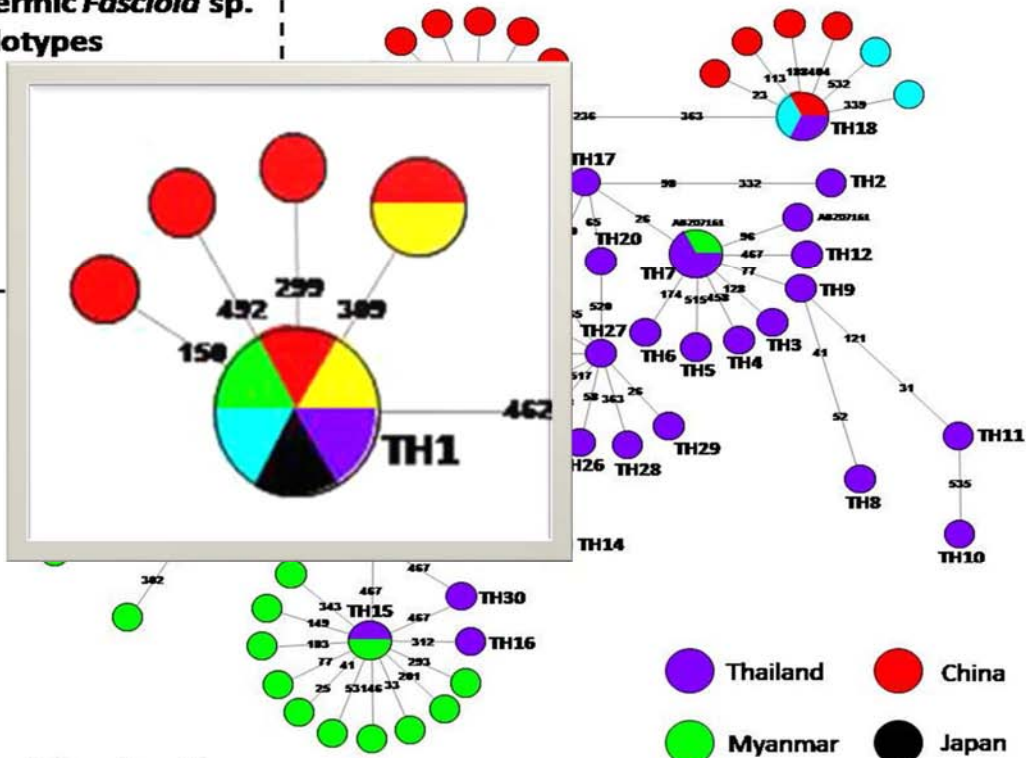
Type	Number of samples	ND1 haplotype
<i>F. gigantica</i>	128	29 (FgTH2-30)
Parthenogenetic <i>Fasciola</i> sp.	18	1 (FgTH1)

Aspermic *Fasciola* sp. haplotypes



Median joining network for of 30 haplotypes from Thailand with other countries

Aspermic *Fasciola* sp. haplotypes

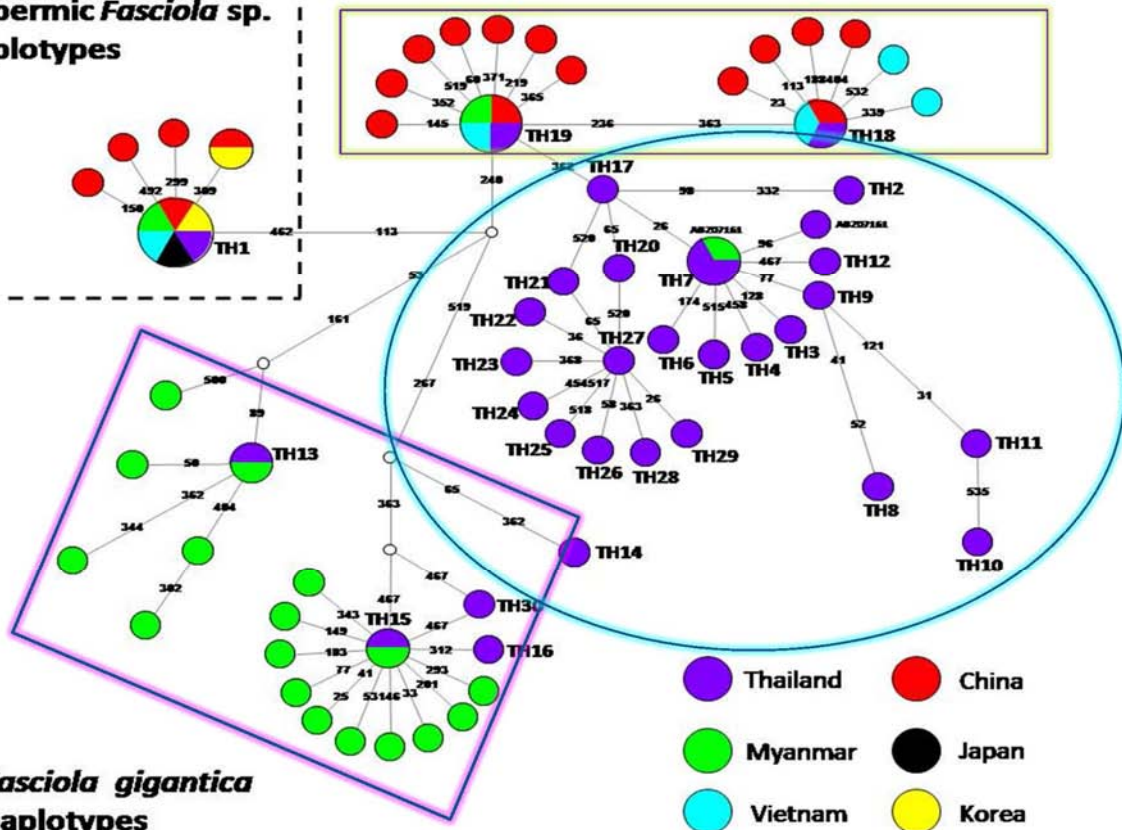


***Fasciola gigantica* haplotypes**



Median joining network for ND1 haplotypes from Thailand with other countries

Aspermic *Fasciola* sp. haplotypes



***Fasciola gigantica* haplotypes**



Median joining network for ND1 haplotypes from Thailand with other countries

Discussions

- *Aspermic Fasciola populations in Thailand* appeared to be genetically identical since all of the aspermic flukes showed the identical genotype (Fg type) in ITS1 and haplotype (Fg-ND1-Thai 1) in ND1, suggesting that they are a descendant population derived from a common aspermic ancestor.
- *Spermic F. gigantica* is widely distributed in South and Southeast Asia, DNA data on nuclear ribosomal ITS1 and mitochondrial ND1 are restricted to liver flukes from Vietnam, China and Thailand this study.
- *Network analysis* indicated that the ancestral haplotype of *F. gigantica* investigated in this study might be Fg-ND1-Thai 19 because basic branches of other haplotypes were connected with this haplotype.
- *Fg-ND1-Thai 13, 15, 16 and 30* belonged to haplotype populations previously found only in *F. gigantica* from Myanmar. This finding suggests that *F. gigantica* populations from Thailand and Myanmar are partially related.

Thank you

