

# MOLECULAR VARIATION OF *TAENIA SOLIUM* IN THE WORLD

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**Abstract.** Complete nucleotide sequences of the mitochondrial cytochrome *b* (Cytb) and cytochrome *c* oxidase subunit 1 (CO1) genes from various isolates of *Taenia solium* were examined. Eleven isolates were analyzed; two isolates from China, two isolates from Indonesia, one isolate each from India, Thailand, Mexico, Ecuador, Peru, Mozambique and Tanzania. In both genes, two isolates from Indonesia shared the same sequences. Similarly, the isolate from Mexico shared same sequences with that from Peru, and the isolate from Mozambique shared same sequences with that from Tanzania. Phylogenetic trees inferred from different mitochondrial genes yielded almost the same topology. Both the UPGMA and NJ-trees were also very similar. These trees indicate that *T. solium* may be diverged to 2 genetic groups; isolates from Asia form one group and isolates from Africa and Latin America belong to the other. It seems that *T. solium* prevalent in Africa and in Latin America shares the related origin and has recently been introduced to each area, perhaps with domestic pigs or human.

## INTRODUCTION

Cysticercosis is one of the most serious larval cestodiasis of medical importance in humans and of economic importance in pigs. Now cysticercosis is spreading worldwide, especially in Asia, Africa and Latin America. In order to control the parasitic disease, it is important to know the extent of intraspecific variations because variants may differ in their infectivity or pathogenicity to man and to domestic animals. However, intraspecific variation of *T. solium* has been rarely discussed.

With the development of molecular biology, the sequences of various genes have been used for molecular phylogenetic study. Mitochondrial DNA (mtDNA) is considered one of the best neutral markers for revealing phylogenetic relationships among related groups. This is because mitochondria are maternally inherited, mtDNA evolves fairly rapidly, most of the nucleotide substitutions occur at neutral sites, and mtDNA is unlikely to be responsible for morphological changes (Osawa *et al.*, 1999).

In the present study, we examined complete sequences of the mitochondrial cytochrome *b* (Cytb) and cytochrome *c* oxidase subunit 1 (CO1) genes from several isolates of *T. solium*, and inferred the phylogenetic relationships within *T. solium* in the world.

## MATERIALS AND METHODS

### Parasite materials

Eleven isolates of *T. solium* collected from various countries were examined. Six isolates were collected

from Asia, that is, two from China (TsolCHI1, TsolCHI2), two from Indonesia (TsolIRI1, TsolIRI2), one from India (TsolIND), and one from Thailand (TsolTHA). Three isolates were collected from Central and Latin America, one each from Mexico (TsolMEX), Ecuador (TsolECU), and Peru (TsolPER). Two isolates were collected from Africa, one from Mozambique (TsolMOZ) and one from Tanzania (TsolTAN). Geographical origins, hosts and stages of the isolates analyzed are presented in Table 1.

### DNA extraction, amplification and sequencing

We determined the complete sequences of two mitochondrial genes, Cytb and CO1. The lengths of Cytb and CO1 genes are 1,068 bp and 1,620 bp, respectively. DNA from each sample was extracted with commercial available DNA Isolation Kit. DNA sequencing was performed by Dye Terminator Cycle Sequencing Kit and Model 377 DNA Sequencing System.

### Phylogenetic analysis

DNA sequences were aligned for the phylogenetic analyses using the CLUSTAL W computer program (Thompson *et al.*, 1994). The evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980). The phylogenetic tree was constructed by the neighbor-joining (NJ) method (Saitou and Nei, 1997) and UPGMA using the PHYLIP 3.5c phylogeny package (Felsenstein, 1993). The tree was evaluated using the bootstrap test (Felsenstein, 1985) based on 100 resamplings. Phylogenetic trees were outgroup-rooted using the nucleotide sequences from *T. saginata* or *Echinococcus multilocularis*.

Table 1  
*T. solium* isolates examined in this study.

	Origin	Host	stage
TsolCHI1	Henan (China)	Human	Egg
TsolCHI2	Jilin (China)	Human	Proglottid
TsolIRI1	Irian Jaya (Indonesia)	Human	Cysticercus
TsolIRI2	Irian Jaya (Indonesia)	Pig	Cysticercus
TsolIND	India	Pig	Cysticercus
TsolTHA	Thailand	Pig	Cysticercus
TsolMEX	Mexico	Human	Egg
TsolECU	Ecuador	Pig	Cysticercus
TsolPER	Peru	Pig	Cysticercus
TsolTAN	Tanzania	Pig	Cysticercus
TsolMOZ	Mozambique	Pig	Cysticercus

For explanations of abbreviations of samples, see text.

Table 2  
Nucleotide differences between pairs of *T. solium* isolates for the Cytb gene (above the diagonal) and the CO1 gene (below the diagonal).

	TsolCHI1	TsolCHI2	TsolTHA	TsolIND	TsolIRI	TsolECU	TsolPER,MEX	TsolTAN,MOZ
TsolCHI1	-	2	4	4	7	19	19	21
TsolCHI2	1	-	5	5	8	20	20	22
TsolTHA	4	3	-	2	5	17	17	19
TsolIND	3	2	3	-	5	17	17	19
TsolIRI	6	5	8	7	-	21	21	20
TsolECU	17	16	19	18	17	-	3	5
TsolPER,MEX	18	17	20	19	18	5	-	4
TsolTAN,MOZ	15	14	17	16	15	2	3	-

For explanations of abbreviations of samples, see text.

## RESULTS

Throughout the examination of both Cytb and CO1 gene sequences, neither deletions nor insertions were required for multiple alignment. Two isolates from Irian Jaya shared the same sequence. The isolate from Tanzania shared the same sequence with that from Mozambique, and isolate from Peru shared the same sequence with that from Mexico. The number of nucleotide differences detected between pairs of *T. solium* isolates for the Cytb gene and the CO1 genes are presented in Table 2. In both genes, nucleotide differences among Asian isolates are less than 10, whereas those between Asian isolates and American or African isolates are 15 to 21. Differences between American isolates and African isolates are also very small.

Fig 1 shows the phylogenetic trees of *T. solium* isolates inferred from Cytb gene. Both the UPGMA tree and NJ tree yielded almost the same topology. These trees indicate that *T. solium* examined in this study have been separated two distinct groups. Bootstrap values of these groups were very high. Fig 2 shows the phylogenetic trees of *T. solium* isolates inferred from CO1 gene. Topologies of these trees are also almost same as those from Cytb gene, and bootstrap values of these two groups are very high. Phylogenetic relationships among *T. solium*, *T. saginata* and *T. asiatica* inferred from the Cytb and CO1 gene are presented in Fig 3.

## DISCUSSION

In both genes, two distinct clads were recognized

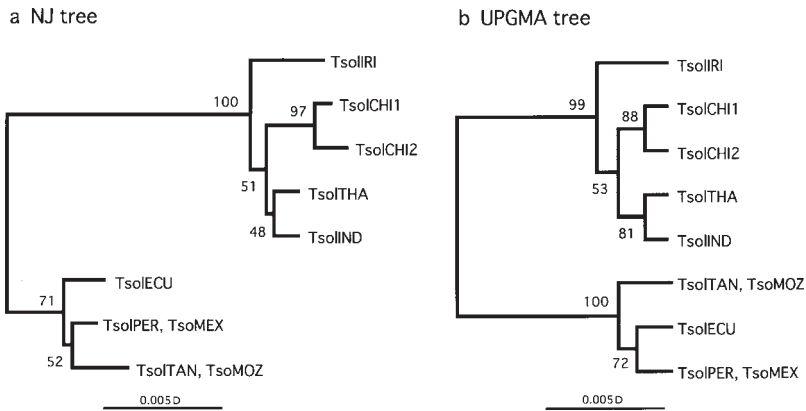


Fig 1- Phylogenetic trees of the *T. solium* isolates inferred from the mitochondrial Cytb gene nucleotide sequences. The values at the nodes represent bootstrap confidence level based on 100 resampling. (a) NJ tree. (b) UPGMA tree.

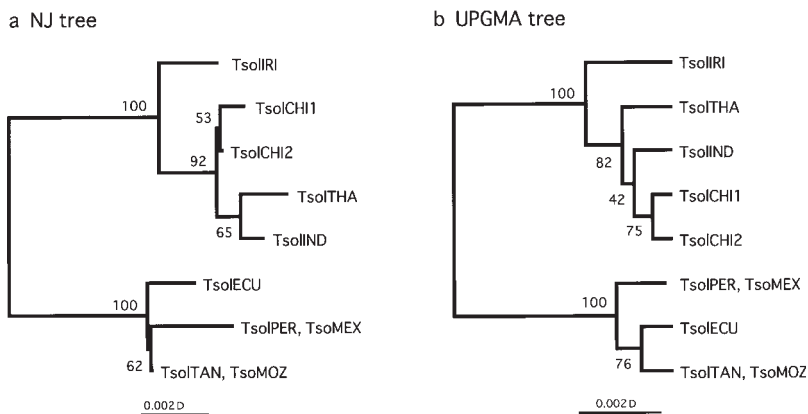


Fig 2- Phylogenetic trees of the *T. solium* isolates inferred from the mitochondrial CO1 gene nucleotide sequences. The values at the nodes represent bootstrap confidence level based on 100 resampling. (a) NJ tree. (b) UPGMA tree.

on both NJ- and UPGMA- phylogenetic trees. It is considered that *T. solium* in the world are separated into two distinct groups, that is, one is the Asian group, another is the American and African group. In the Asian group, isolate from Irian Jaya would have first diverged from the common ancestral line, but the genetic variation within Asian isolates is not so high. In the American and African group, genetic variations within the group are also very small. Although Africa and South America have separated geographically very much, isolates from these areas are closely related. Furthermore, Mexico and Peru are not geographically close. However, isolates from these countries shared same sequences. These facts indicate that those isolates of *T. solium* have been recently introduced into these regions. It seems that *T. solium* prevalent in Africa and Latin America has the same origin and has been introduced with domestic pigs or

humans. Perhaps, the transfer and economic activity of the European peoples might be deeply concerned in this.

Then, how much genetic difference is there between these two groups? It was reported that *T. asiatica* was the related species with *T. saginata*, but biological characteristics of these cestodes were quite different (Fan, 1988). In the CO1 gene, 72 nucleotides differ between *T. saginata* and *T. asiatica*. As mentioned above, nucleotide differences between two groups of *T. solium* are about 20. Fig 3 shows the phylogenetic relationships among *T. solium*, *T. saginata* and *T. asiatica* inferred from the Cytb and CO1 gene. The genetic distance between two groups of *T. solium* was considerably shorter than that between *T. saginata* and *T. asiatica*. The genetic difference between two groups may be very small. In the case of

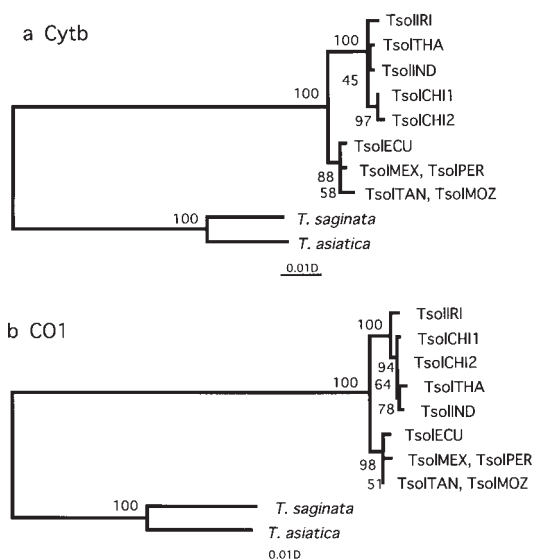


Fig 3- Phylogenetic relationships among *T. solium* isolates, *T. saginata* and *T. asiatica* inferred from the mitochondrial genes. The values at the nodes represent bootstrap confidence level based on 100 resampling. (a) NJ tree inferred from Cytb gene. (b) NJ tree inferred from CO1 gene.

*T. taeniaeformis*, however, it was reported that infectivity of rat strain is quite different from that of mouse strain, although genetic difference between these two strains is very small (Okamoto *et al*, 1995). It is known that cysticercosis in Asia used to occur in the brain and subcutaneous tissues, whereas African and American type used to occur in the brain. It is considered that further investigations, including biological examinations, are necessary to determine the extent of the variation among *T. solium*.

The Cytb sequence (1,068 bp) is shorter than CO1 sequence (1,620 bp), however the number of nucleotide differences in both genes are almost the same. It is indicated that evolutionary rate of Cytb gene in cestoda is higher than that of CO1 gene. In the case of cestoda, Cytb gene is considered to be a more suitable marker for revealing phylogenetic relationships among closely related groups, such as intraspecific variations.

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