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Genetic Diversity of *Taenia asiatica* from Thailand and Other Geographical Locations as Revealed by Cytochrome *c* Oxidase Subunit 1 Sequences

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Abstract: Twelve 924 bp cytochrome c oxidase subunit 1 (*cox1*) mitochondrial DNA sequences from *Taenia asiatica* isolates from Thailand were aligned and compared with multiple sequence isolates from Thailand and 6 other countries from the GenBank database. The genetic divergence of *T. asiatica* was also compared with *Taenia saginata* database sequences from 6 different countries in Asia, including Thailand, and 3 countries from other continents. The results showed that there were minor genetic variations within *T. asiatica* species, while high intraspecies variation was found in *T. saginata*. There were only 2 haplotypes and 1 polymorphic site found in *T. asiatica*, but 8 haplotypes and 9 polymorphic sites in *T. saginata*. Haplotype diversity was very low, 0.067, in *T. asiatica* and high, 0.700, in *T. saginata*. The very low genetic diversity suggested that *T. asiatica* may be at a risk due to the loss of potential adaptive alleles, resulting in reduced viability and decreased responses to environmental changes, which may endanger the species.

Key words: *Taenia asiatica*, *Taenia saginata*, *cox1* gene, genetic diversity, Thailand, different geographical location

INTRODUCTION

Taenia asiatica is one of the 3 species of parasitic tapeworms (*Taenia solium*, *Taenia saginata*, and *T. asiatica*) reported as human pathogens [1,2]. This parasite is distributed in Asian countries where people eat inappropriately cooked viscera of pigs, especially the liver contaminated with metacestodes. *T. asiatica* was first described as a new species, based on morphologic characters of adult worms and metacestodes [3]. Studies on the morphology of adult worms showed that *T. asiatica* is very similar to *T. saginata*; therefore, it is very difficult to distinguish one from the other [4,5]. The 2 species can be discriminated by genetic data such as restriction fragment length polymorphism (RFLP) data and/or mitochondrial DNA sequence data [6-8].

T. asiatica and *T. saginata* diverged from a shared common ancestor around 0.16 million years ago [9]. After separation, these 2 lineages have evolved to have different intermediate hosts in their life cycle. *T. asiatica* infects the visceral organs of pigs while *T. saginata* infects the muscle of cattle [5,10,11]. Moreover, geographical localities between these parasites are different. *T. asiatica* is distributed in specific areas, e.g., in several countries of Asia, including Taiwan [11], Korea [3], China [12], Vietnam [13], Indonesia [14], and Thailand [15], but *T. saginata* can be found worldwide [16].

In Thailand, Thong Pha Phum District, northwest of Kanchanaburi, a west-central province, is the only reported site of *T. asiatica*. It is still unknown if differences in geographical locations and ecological niches affect intra- and inter-specific variations. In this study, we aimed to reveal genetic variations within the species of *T. asiatica* and of *T. saginata* using cytochrome *c* oxidase subunit 1 (*cox1*) as a DNA marker.

MATERIALS AND METHODS

Parasites and source of gene sequences

Taenia proglottids used in this study were previously collected from patients in Kanchanaburi Province during 2003-7. A rough gross morphology of either gravid segment or scolex was identified as *T. saginata* were collected. Worms were preserved in 70% ethanol and kept frozen (-70℃) until used. The scolex, with mature and gravid proglottids from some cases

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Table 1. The *cox1* sequences of 30 *T. asiatica* isolates and 16 *T. saginata* isolates from different geographical localities

Species	No. of isolates	Locality (country)	Accession no.
T. asiatica	4	China	AB465211, AB465212, AB465213, AB465227
	1	Taiwan	AB465230
	2	Korea	AB465224, AB465225
	3	Japan	AB608736, AB608739, AB608742
	1	The Philippines	AB465229
	3	Indonesia	AB465215, AB465216, AB465228
	4	Thailand	AB533174, AB533175, AB465222, AB465223
	12	Thailand, this study	JQ517298-JQ517309
T. saginata	4	China	AB533172, AB533168, AB533169, AB533171
	1	Korea	AB465246
	1	Japan	AB465244
	1	Indonesia	AB465240
	5	Thailand	AB465239, AB533173, AB465247, AB465248, AB465242
	1	Cambodia	AB465241
	1	Ethiopia	AB465245
	1	Ecuador	AB465243
	1	Brazil	AB465238

were fixed with 10% formalin, stained with acetic carmine and examined morphologically. Twelve molecularly identified *T. asiatica* isolate samples were used in this study [15,17,18]. The other sequences of *T. asiatica* and *T. saginata* used for analysis in this study were compiled from the GenBank database (Table 1).

DNA preparation, PCR, and sequencing

Partial fragments of the strobilated proglottids from each isolate were separated and washed with distilled water, to remove any ethanol remaining from the fixation process. The genomic DNA of each worm was extracted using a Genomic DNA Mini Kit (Geneaid Biotech Ltd, Sijhih City, Taiwan), according to the manufacturer's instructions. DNA was re-suspended in 50 µl of elution buffer (provided in the kit). The 1,140 bp PCR amplicons were amplified using 2 oligonucleotide primers: *cox1* (forward); 5´-CATGGAATAATAATGATTTTC-3´ and *cox1* (reverse); 5´-ACAGTACACACAATTTTAAC-3´. These primers were designed from the alignment of *T. saginata* and *T. asiatica* mitochondrial *cox1* genes (AB533171 and AB533175, respectively). PCR amplicons were produced in 50 µl of reaction mixture containing 10 ng genomic DNA, 0.5 µM of each primer, 1X Top*Taq*™ Master Mix Kit (Containing Top*Taq* DNA Polymerase, PCR Buffer (with 1.5 mM $MgCl_2$), and 200 µM each dNTP) (QIAGEN, Hilden, Germany). Amplification conditions were as follows: initial heating at 94℃ for 3 min, followed by 30 amplification cycles, consisting of denaturation at 95℃ for 30 sec, annealing at 53℃ for 30 sec, and elongation at 72℃ for 50 sec. PCR products were run on 1.2% agarose gel and visualized with a UV illuminator. The PCR amplicons were purified and sequenced by dideoxy-termination method and ABI3730XL sequencer and BigDye v3.1 (Applied Biosystems, Foster City, California, USA) at Macrogen Inc. (Geumcheon-gu, Seoul, Korea).

Clustering diagram and Genetic diversity analysis

The *cox1* sequences were aligned by Clustal X version 2.0 [19], and haplotypes were distinguished. A neighbor-joining (NJ) phylogram was constructed under *p*-distance model by MEGA version 5.0 [20]. Bootstrap analyses were conducted using 1,000 replicates. The genetic diversity values including polymorphic sites (*S*), haplotype numbers (*h*), haplotype diversity (*Hd*), and nucleotide diversity (π) were calculated by using the DnaSP 5.0 program [21]. This program was also used to evaluate the genetic structure of the parasites under the population expansion effect via Tajima's *D* test.

RESULTS

We found significant differences in the amount of genetic variations within *T. asiatica* and within *T. saginata* (Table 2). The sequences of the 924 bp *cox1* gene of *T. asiatica* from 12 Thai samples were added. The genetic variations among 30 samples of *T. asiatica* from 7 different countries (China, Taiwan, Korea, Japan, the Philippines, Indonesia, and Thailand) (Table 1) were almost identical (Table 2). Only 2 haplotypes (A and B) of the *cox1* gene were found. The major genotype of

Table 2. Genetic diversity and neutrality values estimated from *cox1* sequences of *T. asiatica* and *T. saginata* population

Species	No. of isolates	*S*	*h*	*Hd*	π	Tajima's *D*[a]
T. asiatica	30	1	2	0.067	0.000	-1.15
T. asiatica (Thai)	16	0	0	0	0	n/a[b]
T. saginata	16	9	8	0.700	0.034	-1.731

S: segregation (polymorphic) site, *h*: haplotype number, *Hd*: haplotype diversity, π: nucleotide diversity.
[a]$P < 0.05$.
[b]Not analyzed.

T. asiatica was haplotype A, composed of 29 samples. Only 1 sample from China was haplotype B, with only 1 polymorphic site found at nucleotide position 115 (Tables 3, 4). On the other hand, for *T. saginata* there were 8 different genotypes (haplotype C to J) with 9 polymorphic sites (Tables 3, 4) in 16 samples from 9 countries in both Asia and other countries (Table 1). The major genotype is haplotype C which is composed of 8 isolates. The nucleotide variations were 1-3 positions in each haplotype. The genetic variation, therefore, in *T. saginata* is high. The segregation site *S* (polymorphic site) and

Table 3. Nucleotide variation sites of partial *cox1* gene (924 bp length) between *T. asiatica* and *T. saginata* from 11 different countries[a]

Species	Nucleotide position[b]	006	027	039	048	060	063	108	115	156	174	177	180	186	204	219	231	237	267	270	324	396	423	444	489	492	505	606	621	624	651	687	729	750	806	816	861	864	891	901	903	909	912	924	Frequency[e]
T. asiatica	Haplotype[c]																																												
	A	G	C	T	C	A	.	A	A	A	.	A	C	G	G	T	.	T	G	A	G	G	A	A	A	.	A	G	.	G	C	T	C	G	.	G	A	G	C	T	A	G	A	.	29
	B	G	C	T	C	A	.	A	**G**	A	.	A	C	G	G	T	.	T	G	A	G	G	A	A	A	.	A	G	.	G	C	T	C	G	.	G	A	G	C	T	A	G	A	.	1
T. saginata	C	A	T	C	T	G	G	G	A	G	G	T	T	A	A	C	A	C	A	G	A	A	G	G	G	A	G	T	G	A	T	C	T	A	C	A	T	A	T	C	G	A	G	T	8
	D[d]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	E[d]	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
	F[d]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	1
	G[d]	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
	H[d]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	1
	I[d]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	A	1
	J[d]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	1

[a]Eleven countries here involved in the genetic analysis include Thailand, Taiwan, Korea, China, Japan, Indonesia, the Philippines, Cambodia, Ecaudor, Brazil, and Ethiopia. The bold G font indicates nucleotide variation of *T. asiatica.*
[b]The numbering of nucleotide positions 1-924 refers to position 400-1,324 of the complete mtDNA sequence (1,620 bp) of *T. asiatica* (GenBank Acc. No. AB465227).
[c]Haplotype A and B are genotypes of *T. asiatica* while haplotype C to J are genotypes of *T. saginata*.
[d]Dots represent homology with haplotype C sequence of *T. saginata*.
[e]No. of samples in each haplotype.

Table 4. The frequency of worm numbers (46) in each haplotype of *T. asiatica* and *T. saginata* from 11 different geographical countries

Species	Haplotype	Frequency											Total
		CHN	TWN	KOR	JPN	PHL	INA	THA	CAM	ETH	ECU	BRA	
T. asiatica	A	3	1	2	3	1	3	16	0	0	0	0	29
	B	1	0	0	0	0	0	0	0	0	0	0	1
T. saginata	C	1	0	1	1	0	1	2	0	1	0	1	8
	D	0	0	0	0	0	0	1	0	0	0	0	1
	E	1	0	0	0	0	0	0	1	0	0	0	2
	F	0	0	0	0	0	0	0	0	0	1	0	1
	G	1	0	0	0	0	0	0	0	0	0	0	1
	H	1	0	0	0	0	0	0	0	0	0	0	1
	I	0	0	0	0	0	0	1	0	0	0	0	1
	J	0	0	0	0	0	0	1	0	0	0	0	1

CHN, China; TWN, Taiwan; KOR, Korea; JPN, Japan; PHL, The Philippines; INA, Indonesia; THA, Thailand; CAM, Cambodia; ETH, Ethiopia; ECU, Ecuador; BRA, Brazil.

haplotype number *h* of *T. asiatica* was 1 and 2, respectively, while those of *T. saginata* was 9 and 8, respectively (Table 2). Significant differences between *T. asiatica* and *T. saginata* were seen when comparing the haplotype diversity: *Hd*, which was 0.067 in *T. asiatica* and 0.700 in *T. saginata*. However, the value of nucleotide diversity: π of each 2 species was very low (Table 2). The genetic variations of *T. asiatica* and *T. saginata* were indicated by genetic divergence on the clustering diagram (Fig. 1).

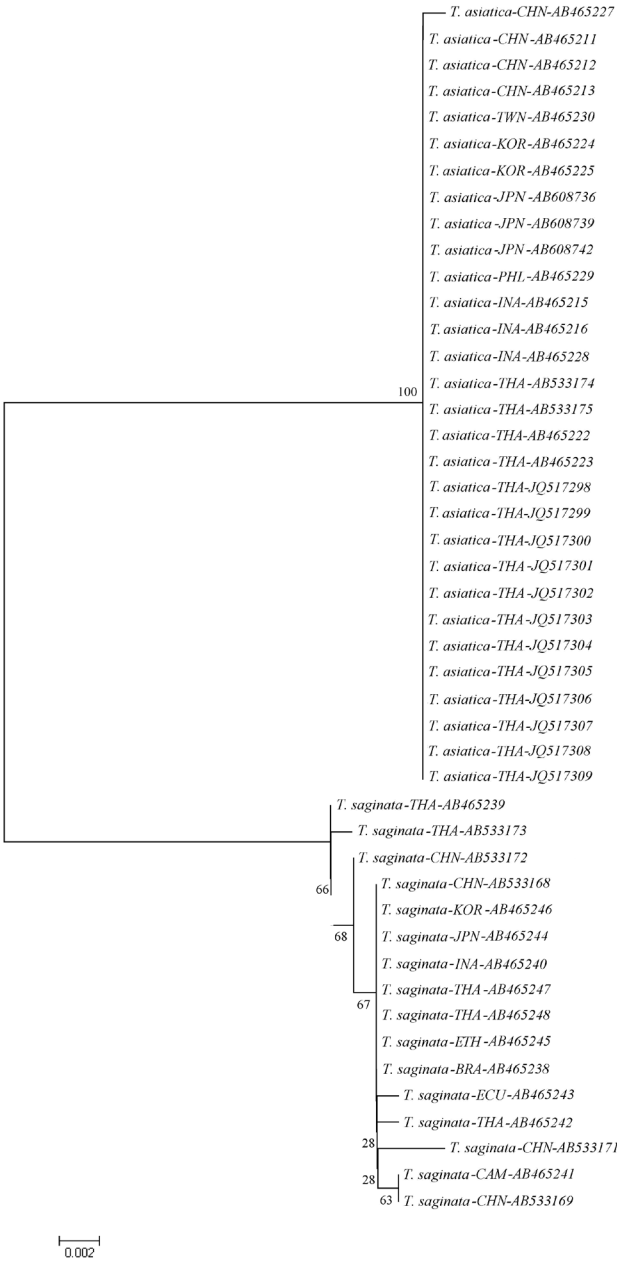

Fig. 1. Phylogenetic tree of *T. asiatica* and *T. saginata* constructed by the neighbor-joining method from genetic distance estimated by the *p*-distance model. The numbers on each internal node is a bootstrap proportion. JQ517298-JQ517309 are samples in this study.

DISCUSSION

Although *T. asiatica* and *T. saginata* derived from a shared common ancestor a long time ago, their morphological characters are still similar [5]. DNA variation is a clear information to clarify their genetic divergence. The mitochondrial genes, *cox1* and cytochrome *b* (*cob*), showed a distant relationship between *T. asiatica* and *T. saginata* [8]. The different intermediate hosts in their life cycle between these species might be one of the conditions causing genetic divergence. The intermediate host of *T. asiatica* is the pig while that of *T. saginata* is cattle [5]. The obvious difference between the farming of pigs and of cattle is population migration. Cattle frequently move from one locality to another to find food, while pigs are restricted to their pigsty.

The phylogenetic tree showed genetic variations of both parasites were not related to geographical locality. For *T. asiatica*, the low genetic variation might suggest that populations of the parasite might be small, i.e., prevalence of *T. asiatica* is low when compared to *T. saginata* in most countries; with very low recombination. For *T. saginata*, the results showed high genetic variations but no specific locality for each genotype. It might suggest that *T. saginata* populations migrated during cattle farming. The gene flow among *T. saginata* might be one effect influenced by population migration. The Tajima's *D* value was tested for gene flow of *T. asiatica* and *T. saginata*. The test revealed no gene flow in both species samples studied. It is possible that the sample size tested for *T. saginata* is small. The results suggested that more samples of *T. saginata* are needed to clarify its population structure. In this study, it is suggested that very low genetic diversity in *T. asiatica* (Table 2) might lead to a reduction of the parasite species, due to loss of potentially adaptive alleles for surviving in changing environments as similarly recognized with other endangered species [22].

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