# Geographical Distribution of *Taenia asiatica* and Related Species

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**Abstract:** Geographical information of *Taenia asiatica* is reviewed together with that of *T. solium* and *T. saginata*. Current distribution of *T. asiatica* was found to be mostly from Asian countries: the Republic of Korea, China, Taiwan, Indonesia, and Thailand. Molecular genotypic techniques have found out more countries with *T. asiatica* from Japan, the Philippines, and Vietnam. Specimens used in this paper were collected from around the world and mostly during international collaboration projects of Korean foundations for parasite control activities (1995-2009) in developing countries.

Key words: Taenia asiatica, Taenia solium, Taenia saginata, geographical distribution, multiplex PCR, cox 1

### INTRODUCTION

*Taenia solium, T. saginata,* and *T. asiatica* are 3 zoonotic tapeworms which induce human infections through pigs and cattle as intermediate hosts. Among them, *T. asiatica* is the last known species found in Asian countries where rural people eat undercooked visceral organs of pigs, i.e., the liver, omentum, serosa, and lung [1-4].

The original description of this tapeworm was based on morphological features of an unarmed rostellum, a large number of uterine buds, and posterior protuberances on the gravid proglottids in the adult stage and wart-like formations on the external surface of the bladder wall in the larval stage (called Cysticercus viscerotropica) [5]. Its morphological similarity to T. saginata evoked taxonomic problems arguing that it should be considered as a subspecies of T. saginata. Genetic studies, however, evidenced the species level difference of this tapeworm [6,7]. Mapping study of Taenia tapeworm mtDNA and followed sequence analysis of full mitochondrial genomes of both tapeworms provided further evidences on the validity of T. asiatica by comparing them with that of T. solium [8-10]. All of these results made us possible to develop molecular tools for differential diagnosis of Taenia tapeworms and for application to the survey works of international collaboration projects.

T. solium and T. saginata distribute worldwide as is well known. On the other hand, T. asiatica is found mostly in Asian countries, such as, Taiwan, the Republic of Korea, Indonesia, China, Thailand, Vietnam, Japan, and the Philippines. During the last 14 years, international collaboration projects for parasite control were done with several different collaboration funds of Korea on which this article and data mostly depends. The projects include Korea-China collaborating project (1st) on control strategies for helminthiases in pilot areas (1995-1999) by Korea Association of Health Promotion (KAHP) and Korea International Cooperation Agency (KOICA), Korea-China collaborative project (2nd) of control strategies for helminthiases in pilot areas (2000-2004) by KAHP and KOICA, intestinal parasite control among primary school children in Lao PDR (2000-2004) by KAHP and KOICA, Korea-Lao PDR collaborative project for control of food-borne trematode infections (especially opisthorchiasis) in Lao PDR (2007-2011) by Korean Foundation for International Healthcare (KFIH) and KOICA, the cooperative project on health promotion of Cambodian school children by intestinal parasite control (2006-2008) by KAHP and KOICA, intestinal parasite control in the southwestern area of Cambodia (2009) by KAHP and KOICA, Korea-Tanzania collaborative project on health promotion through parasite control among school children (2005-2009) by Good Neighbors International (GNI) and KOICA, and Korea-Tanzania collaboration project on neglected tropical disease control in Mwanza (2008-2013) by GNI and KOICA.

The present authors participated in those projects from the

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beginning and were interested in *Taenia* tapeworms, especially on the distribution of *T. asiatica* as well as with other helminthic infections. Beef and pork tapeworms were found from most of the countries which we collaborated together and *T. asiatica* was found from mainland China which was the first record in China (see below). This review paper depends on the data obtained by several parasitological techniques classical or molecular and is going to mention some data on *T. asiatica* from the countries that we had project together as well as the countries which were not subjected to the relevant projects; Japan, the Philippines, Mongolia, Vietnam, Indonesia, Thailand, and Taiwan. Interestingly, *T. asiatica* was found also from specimens of Japan and the Philippines by analyzing them in the laboratory (Details of this will be published elsewhere).

*T. asiatica* is now recognized from Korea, China, Taiwan, Thailand, Indonesia, Vietnam, Japan, and the Philippines thus far. Besides the classical methodologies for parasitological examination, genome map and full sequence of mitochondrial DNA were analyzed prior to develop strong and effective molecular tools for differential diagnosis of the tapeworm species.

# DEVELOPMENT OF TOOLS FOR GENOTYPING

#### Mapping of mtDNA

The whole mitochondrial genomes of T. asiatica and T. saginata were amplified and cloned [10]. Both of them were approximately 14 kb in size. The restriction enzyme map of T. saginata mtDNA was constructed from the restriction fragments: 8, 4, and 2 kb, BglII; 4.5, 3.8, 1.5, and 1 kb, HincII; 5, 4.5, 2, and 1.5 kb, HindIII; 9 and 4 kb, PvuII; 12 and 1.8 kb, XhoI. Enzyme sites were not available for BamHI, EcoRI, EcoRV, KpnI or PstI in T. saginata mtDNA. Between T. asiatica and T. saginata, the migration distances of the mtDNA fragments were observed from 46 fragments, including 12 fragments shared. The sequence divergence between T. asiatica and T. saginata was estimated as much as 4.8%. The full length of the mitochondrial cox1 and cob sequences were 1,620 and 1,068 bp in T. asiatica and T. saginata, respectively. The sequence difference between the 2 species was calculated as 4.6% in the cox1 and 4.1% in the cob genes. Two variant nucleotide positions (0.1% of total length) were detected in the cox1 gene among the 5 T. asiatica isolates from China, the Philippines and Korea, whereas 13 variant nucleotide positions (0.2 to 0.8% of total length) were detected in the 10 T. saginata isolates from China, Ethiopia, France, Indonesia, Japan, Korea, Laos, the Philippines, Taiwan, Thailand and Swiss [11].

#### Complete sequence of T. asiatica mtDNA

The complete T. asiatica mitochondrial genome was 13,703 bp long and composed of 36 genes: 12 protein-encoding (3 subunit of cytochrome c oxidase, cox1, cox2 and cox3; 1 subunit of cytochrome b, cob; 7 subunit of NADH dehydrogenase, nad1, nad2, nad3, nad4, nad4L, nad5 and nad6; and 1 subunit of ATP synthase, atp6), 2 small and large subunit ribosomal RNA, 22 transfer RNA genes and a short non-coding region [8]. The tRNA genes were 61-69 bp long, and the secondary structures of 18 tRNAs had typical clover-leaf shapes with paired DHU arms. However, trnC, trnS1, trnS2 and trnR had unpaired DHU arms that were 7-12 bp in length. The tRNAs that transferred serine lacked a DHU arm. The non-coding region was composed of a short non-coding region of 72 nucleotides with a long non-coding region of 176 nucleotides separated by a trnL1/, trnS2/, trnL2/, trnR/, nad5 gene cluster. The sequence of the cox1 gene between T. asiatica and T. saginata differed by 4.6%, while the T. asiatica cytb gene differ by 4.1% and 12.9% from the cytb genes of T. saginata and T. solium, respectively [8].

# Comparing mtDNA of *T. asiaica* with *T. saginata* and *T. solium*

The protein-coding sequences of T. saginata and T. asiatica contain 10,104 bps and 3,368 codons, while 10,048 bps and 3,349 codons in T. solium [9]. Twelve protein-coding genes of T. saginata and T. asiatica differed by 4.6%, while the overall difference between T. saginata and T. asiatica in the entire mtDNA sequence was 4.6%. Divergences in the mt genomes among the Taenia tapeworms ranged from 3.0% to 27.9%. Average pairwise similarity was about 95% in the functional regions of T. saginata and T. asiatica; and the most variable gene was nad5. Highly conservative regions were found in the subunits of cytochrome c oxidase, cytochrome b, 16S rRNA and the tRNAs. Predicted amino acid sequences of nad5 and cox1 genes exhibited 8.1% and 2.2% differences between T. saginata and T. asiatica, respectively. Two classes of functional regions can be identified in the mitochondrial genome of the 3 Taenia tapeworms: a slow-evolving region of non-synonymous substitution sites that includes tRNAs, rRNAs, and D-loop domains; and a fast-evolving region of synonymous substitution sites that includes *atp6* and *nad6*. The overall sequence difference between T. asiatica and T. saginata was 4.6%, while that between T. saginata and T. solium was 11%. The degree of divergence in mtDNA sequence was estimated using the genetic distance of the cob gene between sister species, congeneric species and confamilial genera [12].

# Primer design for Multiplex PCR to differentiate 3 *Taenia* species

Species-specific forward primers were designed based on the nucleotide sequences of valine transfer RNA and NADH dehydrogenase subunit 2 from *Taenia* species [11]. They were designed to amplify different sized products: (1) Ta4978F, specific for *T. asiatica* (5<sup>'</sup>-GGG TTT AAG TTA TAA ATG TGA TGT-3<sup>'</sup>; nucleotides 4978 to 5001 from GenBank accession number AF445798); (2) Ts5058F, specific for *T. saginata* (5<sup>'</sup>-ACT ACA TTT GGT TTG TTT TTG TAG-3<sup>'</sup>; nucleotides 5058 to 5081 from AY684274); and (3) Tso7421F, specific for *T. solium* (5<sup>'</sup>-CTA GGC CAC TTA GTA GTT TAG TTA-3<sup>'</sup>; nucleotides 7421 to 7444 from AB086-256). The reverse primer was from highly conserved region common to all of these tapeworms: Rev7915 (5<sup>'</sup>-CAT AAA ACA CTC AAA CCT TAT AGA-3<sup>'</sup>; nucleotides 5659 to 5685 from AF445-798, nucleotides 5657 to 5683 from AY684274, and nucleotides 7870 to 7895 from AB086256) [11].

# **GEOGRAPHICAL DISTRIBUTION**

Most applied methodologies were the ones for detection of the eggs from stool specimens in the epidemiological surveys: Kato-Katz, Direct smear, Kato's cellophane thick smear, and Stoll's egg counting method. Formalinized specimens were analyzed selectively when they were the only affordable ones. Adult worms recovered with treatment were observed morphologically anytime when they were available. In case they were only a small part of a proglottid which was not enough for morphological observation, nucleotide sequences or multiplex PCR were applied. When only the eggs were available, multiplex PCR was applied on the fecal samples.

#### The Republic of Korea

Prevalence of human taeniasis was reported since 1915 in Korea. A great number of surveys were executed by many researchers thereafter but most of the surveys did not cover the whole country. Since 1971 nationwide surveys were conducted every 5 years revealing taeniid egg positives of between 0.02 and 1.9% [13]. During the period, *T. saginata* had been considered a dominant species over *T. solium*. The epidemiological profile of these *Taenia* species in humans remained unclear up to recent until Jeon et al. [14] reported distribution pattern of *Taenia* tapeworms in Korea. Morphological examination as well as partial nucleotide sequences of mitochondrial *cox1* and ITS2 (internal transcribed spacer 2) were analyzed for 68 specimens from university or in-

stitute museum collections deposited since 1935 [14]. The specimens were identified as 3 T. solium, 51 T. asiatica, and 14 T. saginata (Table 1) [11,14]. Each province in Korea exhibited 1, 2, or 3 kinds of tapeworms. The distribution ratio of T. asiatica: T. saginata calculated from both morphological and molecular data was approximately 3.5 : 1. Interestingly this ratio is not much different from the estimation by Eom and Rim who predicted 4: 1 according to the eating habit of raw foods [13]. Twenty-nine of the 68 examined specimens were preserved in 10% formalin. Most of the formalin-preserved samples yielded weak or no PCR amplification, but secondary PCR using the PCR products obtained from the first round of PCR amplifications as a template produced made it possible to use PCR product for direct sequencing. The results clearly indicate that all 3 human Taneia tapeworms are distributing together in Korea [14]. T. asiatica is dominating in Korea and the local Korean peoples get this tapeworm by eating undercooked livers and visceral organs of pigs.

#### China

During the period of 1998 and 2002, a total of 19,894 inhabitants belonging to 3 ethnic minorities in Guangxi Province were surveyed for Taenia tapeworms. Total 927 (4.7%) persons discharged proglottids of tapeworms. In 2002, 108 patients were treated and 117 adult tapeworms were obtained from them. Most of worms (n = 108) were found to be T. saginata, and 9 to be T. solium. Nine cases were mixed infection with both worms. Six adult tapeworms collected from 6 persons of the Zhuang minority residing in the southern part of China (Luzhai) were comparatively analyzed and were turned out to be T. asiatica (Table 1) [11,15]. Experimental infections with eggs from the isolate into the pigs produced cysticerci, each with hookletless scolex and with wart-like formations on the external surface of the bladder wall. There were rostellar protrusions on the scolices of the adult worms. Random amplified polymorphic DNA analysis using 3 arbitrary primers produced bands identical to those of the Korean T. asiatica [15]. This minority people likes to eat raw pork and raw pig liver mixed with sour sauce and salted garlic. The Luzhai people have eating habit of raw pig liver in 8.5% (77/902). Sometimes they eat the fresh raw pig liver without any seasoning right after slaughtering the pig. They live very closely with domestic animals sharing the same house. On the first floor, domestic animals and latrines usually share the same room; the second floor the host living room locates. The domestic animals always clean human feces by eating. This is very different from the customs of the Han people, the majori-

No	Species	Sex/age	Year	Locality	Preservation	Genes	Methods
1	T. asiatica	-	1935	Seoul, Korea	10% Formalin	Cox1, ITS	Sequencing
2	T. asiatica	M/10	1971	Chungju (Chungbuk), Korea	10% Formalin	Cox1, ITS	Sequencing
3	T. saginata	-	1977	Korea	10% Formalin	Cox1, ITS	Sequencing
4	T. saginata	M/36	1978	Korea	10% Formalin	Cox1, ITS	Sequencing
5	T. solium	F/50	1979	Uijeongbu (Gyeonggi), Korea	10% Formalin	Cox1, ITS	Sequencing
6	T. asiatica	F/52	1982	Siheung (Gyeonggi), Korea	10% Formalin	Cox1, ITS	Sequencing
7	T. saginata	M/36	1982	Siheung (Gyeonggi), Korea	10% Formalin	Cox1, ITS	Sequencing
8	T. asiatica	M/48	1982	Yongin (Gyeonggi), Korea	10% Formalin	Cox1, ITS	Sequencing
9	T. saginata	M/56	1983	Nonsan (Chungnam), Korea	10% Formalin	Cox1, ITS	Sequencing
10	T. asiatica	M/51	1983	Korea	10% Formalin	Cox1, ITS	Sequencing
11	T. asiatica	F/51	1983	Yeoncheon (Gyeonggi), Korea	10% Formalin	Cox1, ITS	Sequencing
12	T. asiatica	M/55	1983	Korea	10% Formalin	Cox1, ITS	Sequencina
13	T. asiatica	M/46	1984	Korea	10% Formalin	Cox1, ITS	Sequencina
14	T. saqinata	M/45	1984	Pyeonachana (Ganawon), Korea	10% Formalin	Cox1. ITS	Sequencina
15	T. saginata	M/44	1984	Pyeongchang (Gangwon), Korea	10% Formalin	Cox1. ITS	Sequencina
16	T. saqinata	M/64	1985	Haenam (Jeonnam), Korea	10% Formalin	Cox1. ITS	Sequencina
17	T asiatica	-	1986	Korea	10% Formalin	Cox1 ITS	Sequencina
18	T asiatica	M/29	1986	Seoul Korea	10% Formalin	Cox1 ITS	Sequencing
19	T saqinata	-	1988	Korea	10% Formalin	Cox1 ITS	Sequencing
20	T asiatica	-	1988	Jeiu Korea	10% Formalin	Cox1 ITS	Sequencing
21	T asiatica	-	1989	Cheonaiu (Chunabuk) Korea	10% Formalin	Cox1 ITS	Sequencing
22	T asiatica	М	1992	Gimcheon (Gyeongbuk), Korea	Frozen	Cox1, ITS	Sequencing
23	T asiatica	M/64	1992	Gimcheon (Gyeongbuk), Korea	Frozen	Cox1, ITS	Sequencing
24	T asiatica	F/51	1002	Gyeonaai Korea	10% Formalin	Cox1, ITS	Sequencing
25	T asiatica	-	1002	Churcheon (Gangwon) Korea	Frozen	Cox1, ITS	Sequencing
26	T asiatica	M	1996	Waniu (Jeonbuk), Korea	Frozen	Cox1, ITS	Sequencing
20	T asiatica	F	1007	Hwasun (Jeonnam), Korea	70% Ethanol	Cox1, ITS	Sequencing
28	T asiatica	M	1007	Hwasun (Jeonnam), Korea	70% Ethanol	Cox1, ITS	Sequencing
20	T asiatica	F	1007	Hwasun (Jeonnam), Korea	70% Ethanol	Cox1, ITS	Sequencing
20	T asiatica	F	1007	Hwasun (Jeonnam), Korea	Frozen	Cox1, ITS	Sequencing
31	T asiatica	N/	1007		70% Ethanol	Cox1, ITS	Sequencing
30	T asiatica	M	1007	Voungiu (Gyeongbuk) Korea	70% Ethanol	Cox1, ITS	Sequencing
33	T asiatica	M/57	1007	Wando (Jeonnam), Korea	70% Ethanol	Cox1, ITS	Sequencing
34	T asiatica	E/58	1007	Churcheon (Gangwon) Korea	70% Ethanol	Cox1, ITS	Sequencing
35	T asiatica	F/57	1007	Wando (Jeonnam), Korea	70% Ethanol	Cox1, ITS	Sequencing
36	T asiatica	M/59	1008	Ansan (Gyeonggi), Korea	70% Ethanol	Cox1, ITS	Sequencing
37	T saginata	F/46	1000	Busan Korea	70% Ethanol	Cox1, ITS	Sequencing
38	T. saginata	M/57	2000	Korea	70% Ethanol	Cox1, ITS	Sequencing
30	T asiatica	M/4Q	2000	Cheonan (Chungnam), Korea	Frozen	Cox1, ITS	Sequencing
40	T asiatica	101/45	2000	leiu Korea	70% Ethanol	Cox1, ITS	Sequencing
40 //1	T asiatica	M/43	2000	Secawipo ( Jeju) Korea	70% Ethanol	Cox1, ITS	Sequencing
40 1	T asiatica	F/32	2002	leiu Korea	70% Ethanol	Cox1, ITS	Sequencing
42	T asiatica	N/81	2002	Secawipo ( Jeju) Korea	70% Ethanol	Cox1, ITS	Sequencing
40	T asiatica	E/48	2002	leiu Korea	70% Ethanol	Cox1, ITS	Sequencing
44 45	T. asialica T. colium	E/25	2002	Koroa	10% Europhin	Cox1, ITS	Sequencing
4J 46	T. solium	1/2J	-	Korea	10% Formalin	Cox1, ITS	Sequencing
40	T. Solium T. coginata	101/19	-	Vongin (Gyoonggi), Koroo	10% Formalin	Cox1, ITS	Sequencing
47	T. sayinala T. sociation	- -	1001	Koroa	Frozon	Cox1, ITS	Sequencing
40	T asiatica	NA I	1001	loungpycong (Chunghuk) Koroa	Frozon	Cox1, ITS	Sequencing
49 50	T asialica	IVI NA	1991	Koroo	Frozon	Covi ITC	Sequencing
50	T asialica	IVI	-	Social Koros	Frozon	Cox1, 110	Sequencing
50	T asialica	-	-	Scoul Karaa	Frozon	Cox1, 110	Sequencing
52	T asialica	-	-	Secul Korea	FIUZEII	Cox1, 115	Sequencing
00 54	T. asialica	IVI N 4	-	Jeoui, Korea	FIOZEN	Cox1, IIS	Sequencing
04 55	T. asialica	IVI N 4	1909		FIUZER	Cox1, IIS	Sequencing
55	i.asialica	IVI	-	Seoul, Korea	FIOZEN	COX 1, 115	Sequencing

 Table 1. Taenia tapeworm specimens examined by morphological characterizing and DNA genotyping<sup>a</sup>

(Continued to the next page)

Table 1.	(Continued	from the	previous	page)

No	Species	Sex/age	Year	Locality	Preservation	Genes	Methods
56	T. asiatica	М	1991	Cheongju (Chungbuk), Korea	Frozen	Cox1, ITS	Sequencing
57	T. asiatica	-	-	Korea	10% Formalin	Cox1, ITS	Sequencing
58	T. asiatica	F	1991	Cheongju (Chungbuk), Korea	Frozen	Cox1, ITS	Sequencing
59	T. asiatica	Μ	1992	Cheongju (Chungbuk), Korea	10% Formalin	Cox1, ITS	Sequencing
60	T. asiatica	M/71	-	Korea	10% Formalin	Cox1, ITS	Sequencing
61	T. asiatica	М	-	Korea	10% Formalin	Cox1, ITS	Sequencing
62	T. saginata	M/39	2004	Cheongju (Chungbuk), Korea	Frozen	Cox1, ITS	Sequencing
63	T. asiatica	-	2003	Jeju, Korea	70% Ethanol	Cox1, ITS	Sequencing
64	T. asiatica	-	2003	Jeju, Korea	70% Ethanol	Cox1, ITS	Sequencing
65	T. saginata	M/37	2003	Korea	70% Ethanol	Cox1, ITS	Sequencing
66	T. asiatica	F/65	2004	Chuncheon, Korea	70% Ethanol	Cox1, ITS	Sequencing
67	T. saginata	М	2005	Jeju, Korea	70% Ethanol	Cox1, ITS	Sequencing
68	T. asiatica	M/66	1998	Luzhai, China	Frozen	Cox1, ITS	Sequencing
69	T. asiatica	M/64	1998	Luzhai, China	Frozen	Cox1, ITS	Sequencing
70	T. asiatica	M/55	1998	Luzhai, China	Frozen	Cox1, ITS	Sequencing
71	T. asiatica	M/28	1998	Luzhai, China	Frozen	Cox1, ITS	Sequencing
72	T. asiatica	M/30	1998	Luzhai, China	Frozen	Cox1, ITS	Sequencing
73	T. asiatica	M/18	1998	Luzhai, China	Frozen	Cox1. ITS	Sequencing
74	T. asiatica	M/59	1968	Izumo, Japan	10% Formalin	Cox1	Sequencing
75	T. asiatica	M/41	1996	Yonago, Japan	10% Formalin	Cox1	Sequencing
76	T. asiatica	M/40	1970	Samosir, Indonesia	10% Formalin	Cox1	Sequencing
77	T asiatica	F/40	1982	Samosir Indonesia	10% Formalin	Cox1	Sequencing
78	T asiatica	-	-	Taiwan	Frozen	Cox1	Sequencing
79	T asiatica	_	-	Taiwan	Frozen	Cox1	Sequencing
80	T sacinata	_	-	Taiwan	Frozen	Cox1	Sequencing
81	T saginata	_	1991	Thailand	Frozen	Cox1	Sequencing
82	T asiatica	_	-	Manila Philippine	Frozen	Cox1	Sequencing Multiplex PCB
83	T sacinata	_	-	Manila, Philippine	Frozen	Cox1	Sequencing Multiplex PCB
84	T saginata	_	-	Beijing China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
85	T saginata	_	1999	Guangxi China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
86	T saginata	_	1999	Guangxi, China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
87	T saginata	_	1999	Guangxi, China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
88	T saginata	_	-	Henan China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
89	T solium	_	1998	Luzhai China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
90	T solium	_	1000	Guangyi China	70% Ethanol	Cox1	Sequencing, Multiplex PCR
91	T solium	M/28	1998	Tiandong China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
92	T solium	M/31	1998	Tiandong, China	70% Ethanol	Cox1	Sequencing, Multiplex PCR
92	T solium	-	-	Sanijang, China	70% Ethanol	Cox1	Sequencing, Multiplex PCB
9/	T solium	_	2000	Nei Mongu, China	70% Ethanol	Cox1	Sequencing, Multiplex PCR
95	T saninata	E/51	2000	Mongolia	70% Ethanol	Cox1	Sequencing, Multiplex PCB
96	T saginata	F/40	2000	Savanakhet Laos	Frozen	Cox1	Sequencing, Multiplex PCR
97	T saginata	F/48	2002	Savanakhet Laos	Frozen	Cox1	Sequencing, Multiplex PCB
98	T saginata	F/53	2002	Savanakhet Laos	Frozen	Cox1	Sequencing, Multiplex PCB
90	T saginata	M/28	2002	Khammouane Laos	Frozen	Cox1	Sequencing, Multiplex PCB
100	T saginata	M/18	2000	Khammouane, Laos	Frozen	Cox1	Sequencing, Multiplex PCR
100	T. sayinala T. saqinata	M/34	2003	Khammouane Laos	Frozen	Cox1	Sequencing, Multiplex PCR
101	T. saginala T. coginata	M/42	2003	Khammouano, Laos	Frozon	Cox1	Sequencing, Multiplex I CR
102	r. sayırıala T. saqinata	F//2	2003	Khammouane Laos	Frozen	Covi	Sequencing, Multiplex FOR
103	r. sayırıald T. saqinata	1/42 F/30	2003	Khammouane Laos	Frozen	Covi	Sequencing, Multiplex PCP
104	r. sayırıald T. səqinətə	N/00	2003	Khammouane Loos	Frozen	Covi	Sequencing, Multiplex PCP
100	i . sayii lald T saginata	1V1/23	2003	Khammouana Laos	Frozon	Covi	Sequencing, Multiplex PCR
100	T socioato	1/41 M/50	2003	Khammouana Laas	Frozen	Covi	Sequencing, Multiplex PCR
107	i. sayinala T. saginata		2003	Khammouana Laas	FIUZEII	Coxt	Sequencing, Multiplex PCR
100	i. sayinala T. saginata	Г/4U M/65	2003	Khammouana Laas	FIUZEII	Coxt	Sequencing, Multiplex PCR
110	T. sayırıala T. oocinata		2003	Khammeurana Laas	FIUZEII	Coxi	Sequencing, Multiplex PCR
ΠU	i. saginata	IVI/55	2003	Knammouane, Laos	Frozen	COXI	Sequencing, Multiplex PCR

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Table 1. (Continued from the previous page)

No	Species	Sex/age	Year	Locality	Preservation	Genes	Methods
111	T. saginata	-	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
112	T. saginata	M/10	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
113	T. saginata	F/12	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
114	T. saginata	M/19	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
115	T. saginata	M/27	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
116	T. saginata	F/17	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
117	T. saginata	F/54	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
118	T. saginata	M/19	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
119	T. saginata	F/16	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
120	T. saginata	M/40	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
121	T. saginata	F/44	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
122	T. saginata	M/50	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
123	T. saginata	M/52	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
124	T. saginata	F/36	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
125	T. saginata	F/24	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
126	T. saginata	F/35	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
127	T. saginata	M/28	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
128	T. saginata	F/59	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
129	T. saginata	M/48	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
130	T. saginata	F/28	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
131	T. saginata	F/42	2007	Khokong, Cambodia	Frozen	Cox1	Sequencing, Multiplex PCR
132	T. solium	M/15	2006	Mbulu, Tanzania	Frozen	Cox1	Sequencing, Multiplex PCR
133	T. saginata	M/15	2007	Izaka, Tanzania	Frozen	Cox1	Sequencing, Multiplex PCR
134	T. saginata	M/40	2007	Izaka, Tanzania	Frozen	Cox1	Sequencing, Multiplex PCR
135	T. saginata	M/14	2007	Izaka, Tanzania	Frozen	Cox1	Sequencing, Multiplex PCR
136	T. saginata	M15	2007	Izaka, Tanzania	Frozen	Cox1	Sequencing, Multiplex PCR
137	T. solium	-	2001	Cape Verde	70% Ethanol	Cox1	Sequencing, Multiplex PCR
138	T. saginata	-	-	Ethiopia	70% Ethanol	Cox1	Sequencing
139	T. saginata	-	2003	Chile	70% Ethanol	Cox1	Sequencing
140	T. solium	-	2001	Honduras	70% Ethanol	Cox1	Sequencing
141	T. saginata	-	1999	France	70% Ethanol	Cox1	Sequencing
142	T. saginata	-	1997	Poland	70% Ethanol	Cox1	Sequencing
143	T. saginata	-	1929	Switzerland	10% Formalin	Cox1	Sequencing
144	T. saginata	-	1931	Switzerland	10% Formalin	Cox1	Sequencing
145	T. saginata	-	1941	Switzerland	10% Formalin	Cox1	Sequencing
146	T. saginata	-	1941	Switzerland	10% Formalin	Cox1	Sequencing
147	T. saginata	-	1941	Switzerland	10% Formalin	Cox1	Sequencing
148	T. saginata	-	1941	Switzerland	10% Formalin	Cox1	Sequencing
149	T. saginata	-	1941	Switzerland	10% Formalin	Cox1	Sequencing
150	T. saginata	-	1941	Switzerland	10% Formalin	Cox1	Sequencing
151	T. saginata	-	1990	Belgium	70% Ethanol	Cox1	Sequencing

<sup>a</sup>Data partly by courtesy of Eom et al. (2002), Jeon et al. (2008), and Jeon et al. (2009).

-, unknown.

ty group, who cook meat thoroughly in boiling oil (Korea Association of Health Promotion and Korea International Cooperation Agency, 2004; Final report on the Korea-China collaborative project of control strategies for helminthiasis in pilot areas, 2000-2004).

Reports of other researchers give more information on the distribution of *T. asiatica* by finding out 2 cases from Lanping County, Yunnan Province which was confirmed by morphological observation and experimental infection in intermediate

host animals [16]; Dali of Yunnan Province and Duyun of Guizhou Province by mitochondrial *CoxI* analysis of the worms [17]; and 3 cases from Tibet of Sichuan by DNA genotyping [18].

#### Japan

In December of 1998, Dr. Yosuke Yamane provided 3 *Taenia* tapeworms to the author's laboratory for a Christmas gift. The worms were kept in 10% formalin and each was attached with questionnaire sheet for detailed information asking about food

and identification data. Two specimens were labeled as *Taeni-arhynchus saginatus*, one was from inhabitant in Izumo City (male, 59-year-old) and the other from Yonago City (male, 41-year-old), and both of them were identified as *T. asiatica* by DNA genotyping (Table 1). PCR amplification and direct sequencing for the *cox1* target fragment (349 bp in length corresponding to the positions 80-428 bp of the *cox1* gene) were performed using the total genomic DNA extracted from formalin-preserved samples. PCR amplification was successful in these cases and generated high quality PCR products applicable to direct sequencing. This is the first report of this tapeworm from Japan (Details of this will be published elsewhere).

#### The Philippines

A case of T. asiatica was found from out of the 2 specimens examined by nucleotide sequencing of Cox1 and multiplex PCR (Table 1) [11]. The other was T. saginata. Leon commented that Taenia segments, which were identified morphologically as T. saginata, were examined for mitochondrial DNA through the courtesy of Drs. A. Ito and H. Yamasaki and finally resulted in 5 T. asiatica out of the 6 Filipino specimens [19]. Fan et al. [20] had a result of successful experimental infection in Small-Ear Miniature pigs with Taenia eggs from the Philippines. Hinz [21] already had stated that "In the Philippines the infections with T. saginata is clearly dominated in man but the extremely rare T. saginata cysticercosis in cattle and carabao constitutes a still unresolved epidemiological paradox for the Philippines. In general neither beef nor the carabao meat is often eaten by the population of the endemic area. In endemic area 92.6% of those asked, however, indicated that they ate raw pork (local food prepared as "kinilaw" or "sinugba"). The cycle of T. saginata in the endemic area did not follow the known cycle of man-Bovidaeman. In consideration of our results, we believe we are dealing with a T. saginata-like tapeworm [21]."

#### Taiwan

*T. asiatica* used to be called "Taiwan *Taenia*". Two of our collection demonstrated this tapeworm also with analysis of *Cox1* sequencing (Table 1) [11]. Taiwanese Dr. P.C. Fan made Orchid Island (Lanyu Island) very well known among cestodologists and parasitologists while he was pioneering the research works on Taiwan *Taenia*, as one of the endemic areas of the tapeworm as well as the mainland Taiwan. This small island is a land of the Yami tribe who moved from South-East Asia via the Philippines 2 hundred years ago. The natural intermediate

host of *T. asiatica* was also confirmed in Lanyu native pigs for the first time. Yeh et al. [22] described that "Food-borne parasitic zoonosis such as infections with *Angiostrongylus cantonensis, Clonorchis sinensis,* and *T. saginata asiatica* (*Taenia asiatica*) are not rare, but the former is seasonal and the latter 2 are ethnically and geographically associated". *T. saginata* and *T. solium* are also prevalent in Taiwan by consuming undercooked beef or pork.

#### Indonesia

Cox1 sequencing identified 2 specimens of our collection, both from Samosir Island in Indonesia, as T. asiatica (Table 1) [11]. This tapeworm species is already well known and the country is one of the most endemic areas of taeniasis/cysticercosis. The majority of the people are moslems, but christians predominate in East Indonesia and Hindus in Bali. The 3 major endemic areas of the taeniasis/cysticercosis in Indonesia are North Sumatra, Bali and Papua (former Irian Jaya). Endemic areas are also found in other islands, such as Timor, Flores, North Sulawesi, West Kalimantan and South Sumatra. Inhabitants of Bali eat pork and beef and cysticercosis is common. Approximately 23% of pork liver samples in Bali were found to contain T. asiatica metacestodes [23]. A total prevalence was as high as 13.0% (19/ 146) for T. solium taeniasis in Jayawijaya District, Papua. A 2003-2006 survey of 371 local people in Samosir Island, North Sumatra revealed 6 of 240 (2.5%) to be infected with T. asiatica: 2 of 58 (3.4%) and 4 of 182 (2.2%) cases in 2003 and 2005, respectively [24]. T. asiatica is well known in North Sumatra, especially Samosir Island in Lake Toba. T. solium and T. saginata are well known from Bali [24]. Indonesia is one of the countries which are endemic with all 3 species of human Taenia tapeworms: T. solium, T. asiatica and T. saginata.

#### Thailand

A specimen of our collection was identified as *T. saginata* by *Cox1* nucleotide sequencing but sympatric distribution of *T. solium*, *T. saginata* and *T. asiatica* is already reported from Thailand in 2007 by Anantaphruti et al. on the basis of mitochondrial DNA analysis [11,25]. This was the first report of *T. asiatica* in this country. During 2002-2005, the field investigation was conducted in Thong Pha Phum District of Kanchanaburi Province which was northwest area of Thailand close to Myanmar border. Karen, a tribe, was the most surveyed population where Mon and Thai minorities reside in the mountainous terrain. Total 6 specimens, most of them had scoleces when recovered,

were turned out to be *T. asiatica* by *Cox1* gene analysis. All of them had been considered as being *T. saginata* morphologically before DNA genotyping. The authors stated their study indicated that 53.3% (8/15) of taeniid specimens expected to be *T. saginata* were actually *T. asiatica* and that both *T. asiatica* and *T. saginata* were co-distributed in Kanchanaburi Province. A dual infection of *T. solium* and *T. asiatica* from a patient was also confirmed clearly. In the local area, raw or inadequately cooked beef, pork, or pig viscera, and fresh blood are commonly consumed by local people in the study areas [25].

#### Vietnam

*Taenia* specimens are not available in the authors' laboratory but reports are now available from other researchers. Somers et al. [26] reported that *T. asiatica* (*T. saginata asiatica*) was the most common species (55.4%) over the *T. saginata* (38.5%) and *T. solium* (6.2%) out of 65 *Taenia* samples collected from patients in a referral hospital in Hanoi, North Vietnam. Species identification was done with morphological and molecular techniques: PCR-RFLP of a mitochondrial 12S rDNA [26]. Willingham and his coworkers also reported in 2003 that surveys for human taeniasis in central and northern provinces indicated a prevalence of 0.2-7.2%. In addition to *T. solium* and *T. saginata*, *T. asiatica* is also known to be present in Vietnam [27]. Xuan and colleagues also commented that *T. asiatica* was recently detected in Vietnam [28].

#### Mongolia

A specimen of Taenia species from 51-year-old female Mongolian was given to our laboratory by Dr. Hong Sung-Tae (Seoul National University College of Medicine, 2003). Cox1 sequence analysis and multiplex PCR genotyping classified it as T. saginata (Table 1). Since Mongolian people do not prefer eating raw pork, most of the tapeworms found in the country is T. saginata (personal communication with Dr. D. Temuulen, Health Sciences University of Mongolia School of Biomedicine, 2009). In the years 2002-2006, surveys on taeniasis/cysticercosis was conducted in Mongolia; the 118 proglottids were confirmed to be T. saginata by mitochondrial DNA analysis using cytochrome c oxidase subunit 1 (Cox1) and cytochrome b genes. T. saginata taeniasis was widely distributed at least in 10 of 21 provinces. No variation in the nucleotide sequences of the 2 genes was observed among T. saginata. There was no evidence of T. solium taeniasis/cysticercosis nor T. asiatica taeniasis so far [29].

#### Lao PDR

Total 15 specimens, 3 from Savanakhet and 12 from Khammouane, of Taenia species from our collection were analyzed by Cox1 sequence and multiplex PCR represented all of them as being T. saginata (Table 1) [11]. During the years between 2000 and 2008, helminthiases were surveyed nationwide which were funded by organizations KAHP, KOICA and KFIH. Total 37,090 subjects from 18 localities were examined for helminth eggs. Saravane revealed the highest prevalence (3.0% out of 2,869) of taeniasis over the average of 1.1% throughout the nation. Among 120 collected tapeworms by morphological and genotypical examination, 3 T. solium infections were identified from Luangprabang for the first time in the country. A male patient with neurocysticercosis was also found from Oudomxay in northern territory. A pig with T. solium metacestodes was also found from the same district. The official inspector had data of cysticerci as much as 0.59% (46/7826) in pigs, and 2.99% (44/ 1473) in cattle (2003-2004). Regarding the possibility of T. asiatica in Lao PDR, Phoumindr commented that "Since the neighboring countries like Thailand and Vietnam have it, T. asiatica probably exists in Laos" [30].

#### Cambodia

Total 21 *Taenia* specimens from Khokong in Cambodia were identified as being *T. saginata* by *Cox1* sequencing and multiplex PCR (Table 1). A total of 280 stool samples were examined for helminth ova from 2007 to 2008. The overall prevalence of taeniasis was 21.7% (61/280). We performed molecular epidemiological survey on *Taenia* tapeworms by analysis of copro DNA in Kohkong. For DNA differential diagnosis of *T. solium, T. saginata* and *T. asiatica* eggs, multiplex PCR was used based on the *nad5* gene analysis. Using oligonucleotide primers Ta71-26F, Ts7313F, Tso7466F, and Rev7915, the multiplex PCR was useful for species identification based on the 706, 629, and 474 bp bands. Cambodia still remains unclear for *T. asiatica*.

#### Tanzania

*T. solium* (n = 1) and *T. saginata* (n = 4) were identified by *Cox1* sequencing and multiplex PCR which were collected from Mbulu and Ijaka, Tanzania (Table 1). Fecal samples were obtained from inhabitants in Kongwa, Dodoma area in February, 2008. Total 929 subjects in Ijaka and Pembamoto with primary school students also were examined for helminth eggs using Scotch tape anal swab and Kato-Katz techniques. The overall prevalence of helminthic infection was 7.2% (67/929). The infection rate of taeniasis was 0.6% (6/929). Species identification of *Taenia* tapeworms was performed by multiplex PCR and nucleotide sequencing of *Cox1* gene on the fecal samples containing eggs. *T. solium* and *T. saginata* were recognized from Ijaka. This area was newly recognized to be endemic for *T. solium* in Tanzania [31]. There was, however, no evidence of *T. asiatica* in Tanzania at the moment.

#### Other countries

Other countries, besides the Asian countries which are expected to be endemic for *T. asiatica*, exhibited either *T. saginata* or *T. solium* or both in morphological observations or DNA genotyping in Ethiopia, Cape Verde, Chile, Honduras, France, Poland, Switzerland, and Belgium.

## CONCLUSIONS

*T. asiatica* is currently distributed in Asian countries, i.e., Korea, China, Taiwan, Indonesia, Thailand, Japan (DNA genotyping), the Philippines (DNA genotyping), and Vietnam (DNA genotyping). Some Asian countries, Lao PDR and Cambodia, or some more countries including Mongolia, are suspected to have endemic foci of *T. asiatica* in their territory-especially for some ethnic groups-but still unclear. Most of the continents other than Asia are also endemic for *Taenia* species and the advanced molecular techniques are expected to be applied more in the field of epidemiology for tapeworms.

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