



# Occurrence of a Hybrid Between *Taenia saginata* and *Taenia asiatica* Tapeworms in Cambodia

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**Abstract:** Human infection with *Taenia asiatica* or a hybrid between *Taenia saginata* and *T. asiatica* has not been reported in Cambodia. We detected for the first time a hybrid form between *T. saginata* and *T. asiatica* in Preah Vihear Province, Cambodia. An adult tapeworm specimen, i.e., 75 cm long strobila without scolex, was expelled from a 27-year-old man after praziquantel medication and purging. It was morphologically indistinguishable between *T. saginata* and *T. asiatica*. Several proglottids were molecularly analyzed to confirm the tapeworm species. The mitochondrial gene encoding cytochrome c oxidase subunit 1 (*cox1*) and nuclear genes encoding elongation factor-1 $\alpha$  (*ef1*) and ezrin-radixin-moesin (ERM)-like protein (*elp*) were sequenced, and a single-allele analysis was performed to confirm the haploid genotype. The results revealed that our sample showed a discrepancy between the mitochondrial and 2 nuclear genes. It possessed homozygous sequences typical of *T. saginata* at *cox1* and *ef1* loci. However, it was heterozygous at the *elp* locus, with 1 allele in *T. asiatica* (*elpA*) and 1 in *T. saginata* (*elpC*), which indicates that it is a hybrid between *T. saginata* and *T. asiatica*. The present results confirmed the presence of a hybrid between *T. saginata* and *T. asiatica* in Cambodia and strongly suggest the existence of also 'pure' *T. asiatica* in Cambodia.

**Key words:** *Taenia saginata*, *Taenia asiatica*, hybrid, allelic study, Cambodia

*Taenia solium*, *Taenia saginata*, and *Taenia asiatica* are 3 tapeworm species that can cause human intestinal infections. They have unique life cycles, taking humans as the only definitive host and pigs or cattle as the intermediate host [1]. *T. asiatica*, which is transmitted by swine (viscera), was first identified in Taiwan and then in South Korea and reported as a distinct species from *T. saginata* in 1993 [1,2]. The distribution of human taeniasis by *T. solium* and *T. saginata* is worldwide, whereas *T. asiatica* is found mostly in Asian countries [3,4].

The possibility of hybridization between *T. asiatica* and *T. saginata* has been raised in previous studies that analyzed samples from several countries, including Thailand, China, the Philippines, and Lao PDR [5-8]. Although the 2 taxa have dis-

tinct biological characteristics, including host specificity, gene exchange between the 2 species was strongly suggested using allelic analysis on mitochondrial and nuclear genetic markers [5]. The existence of *T. solium* and *T. saginata* in Cambodia was confirmed by DNA sequencing of egg-positive fecal samples and adult worms expelled from residents [9,10]. However, there has been no report on the occurrence of *T. asiatica* or a hybrid between *T. saginata* and *T. asiatica* in humans despite its geographical location, surrounded by other countries where *T. asiatica* and hybrids between the 2 species have been found [5-8,11]. In this study, we report a hybrid individual between *T. saginata* and *T. asiatica* in a northern part of Cambodia, bordering Lao PDR, suggesting the occurrence of *T. asiatica* in this country.

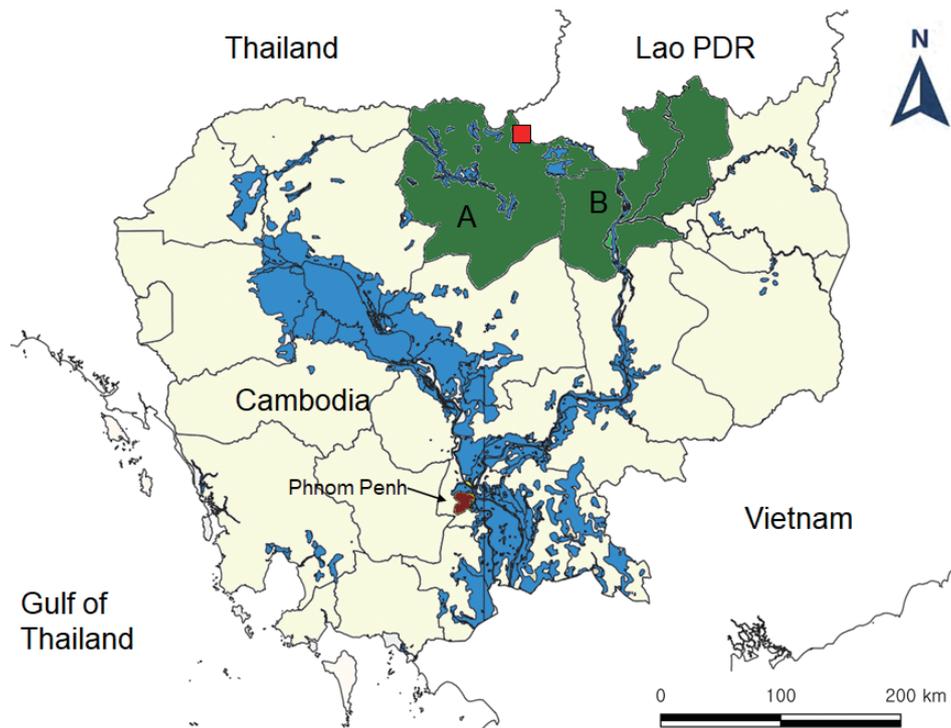
Fecal samples were collected from total 1,156 people in 2 northern provinces (5 villages each), Preah Vihear and Stung Treng (Fig. 1), and examined by the Kato-Katz thick smear technique, as previously reported [10]. The fecal survey of people and worm collection after praziquantel medication were

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**Fig. 1.** Map of the areas for fecal surveys in Cambodia (A, Preah Vihear Province; B, Stung Treng Province) and the village (red, quadrangle) where the present hybrid case between *Taenia saginata* and *Taenia asiatica* was found.

ethically approved by National Ethics Committee for Health Research, Cambodia (IRB no. 099NECHR). Twenty-six (2.4%) out of them were positive for *Taenia* spp. eggs, and 3 tapeworm strobilae were collected from 3 patients in Preah Vihear after praziquantel medication (15 mg/kg single dose) and purging with MgSO<sub>4</sub>.

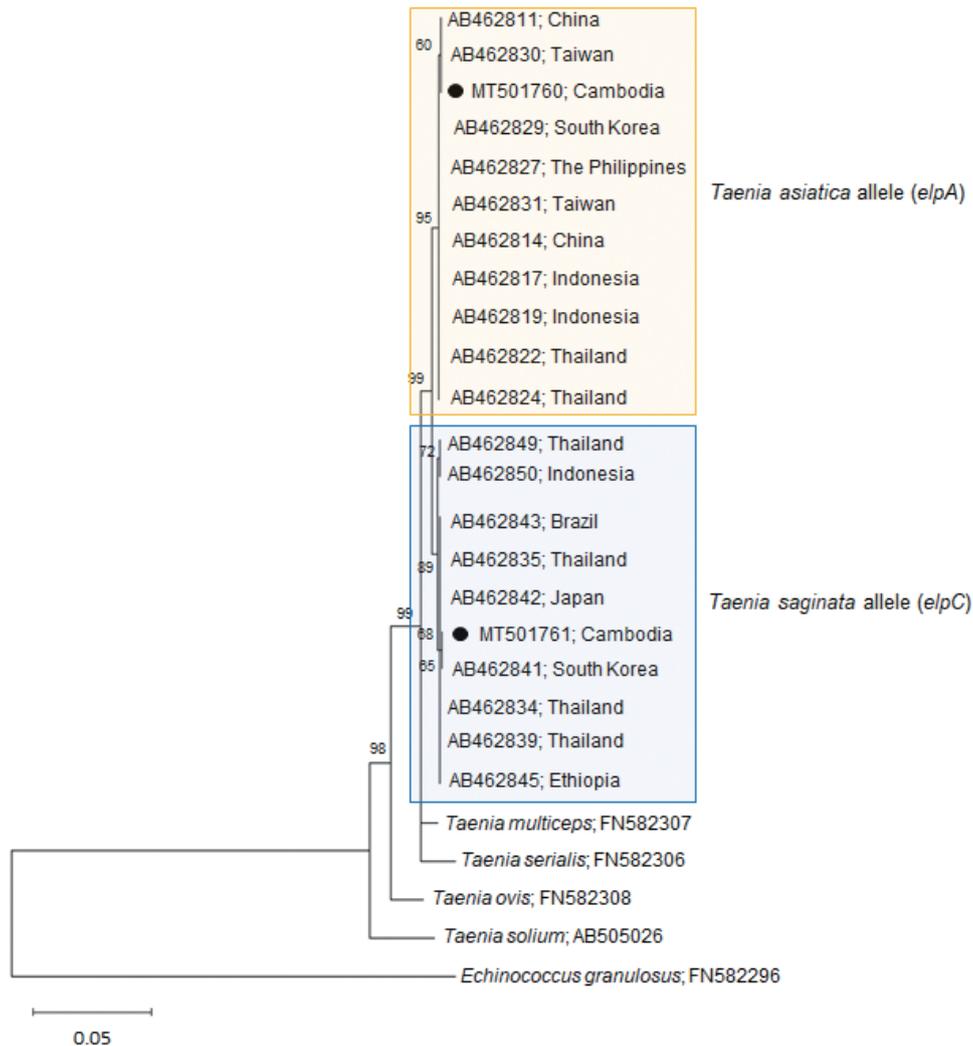
Two of the 3 strobilae were diagnosed molecularly as ‘pure’ *T. saginata* based on the mitochondrial gene encoding cytochrome c oxidase subunit 1 (*cox1*), and 2 nuclear genes encoding elongation factor-1 $\alpha$  (*ef1*) and ezrin-radixin-moesin (ERM)-like protein (*elp*) which were reported previously [10]. The remaining 1 strobila (75 cm in length, mostly gravid proglottids without a scolex) expelled from a 27-year-old man showed strange results that the *elp* allele type was different from ‘pure’ *T. saginata*. In this study, we further analyzed the allele type of the *elp* gene of this strobila. The sample preparation and genomic DNA extraction were conducted as described in our previous study [10]. The analyses of mitochondrial *cox1* (1,620 bp) and 2 nuclear genes *ef1* (1,090 bp) and *elp* (1,160 bp) were performed following conditions of Okamoto et al. [5]. A consistent sequence data of *elp* locus could not be obtained since double peaks were observed in electro-

pherograms. Thus, to confirm the haploid genotype of *elp* locus, PCR amplification and sequencing were conducted at the single-allele level using T-Blunt PCR cloning kit (Solgent, Seoul, Korea).

Multiple sequences alignment was constructed with Clustal W [12]. A phylogenetic tree of *elp* locus sequences identified in this study and representative sequences of *Taenia* spp. available in the GenBank database were constructed using the maximum-likelihood (ML) method based on the Tamura-Nei model of nucleotide substitution. The tree was viewed by MEGA v6 [13]. Bootstrap values were calculated with 1,000 replications.

Our *Taenia* strobila sample showed a discrepancy in the diagnosis between the mitochondrial and nuclear genes. It possessed homozygous sequences typical of *T. saginata* *cox1* (MT074050) and *ef1* (MT075318) loci. However, at the *elp* locus, it was heterozygous with 1 allele (MT501760) matched with *T. asiatica* (*elpA*) and another (MT501761) with *T. saginata* (*elpC*) (Table 1; Fig. 2).

The homology between our allele sample *elpA* and *T. asiatica* (AB462829-AB462830) was 99.9-100%, whereas the homology between our sample *elpA* and *T. saginata* (AB462841-AB462842) or *T. solium* (AB505026) was lower than that, 99.1-



**Fig. 2.** A phylogenetic tree of our sample (alleles) in comparison with related *Taenia* spp. tapeworms drawn with *elp* DNA sequences using the maximum-likelihood method employing Tamura-Nei model of nucleotide substitution with 1,000 bootstrap replications. Black dots (●) indicate the sequences identified in this study. The allele MT501760 has been registered under the name *T. saginata* in GenBank; however, this allele is actually classified as a *T. asiatica* allele. Scale bar indicates nucleotide substitutions per site.

**Table 1.** Genotypes of the *Taenia* tapeworm sample from our patient

Patient	Mitochondrial gene ( <i>cox1</i> )	Nuclear genes	
		Genotype at <i>ef1</i> locus <sup>a</sup>	Genotype at <i>elp</i> locus <sup>a</sup>
27-year-old male	<i>T. saginata</i> type	<i>ef1C/ef1C</i> ( <i>T. saginata</i> )	<i>elpA/elpC</i> ( <i>T. asiatica/T. saginata</i> hybrid)

<sup>a</sup>The allele types of the sample were categorized following Okamoto et al. [5].

99.2% or 94.7%, respectively. In addition, the homology between our allele sample *elpC* and *T. saginata* (AB462841-AB462842) was 99.9-100%, whereas the homology between our sample *elpC* and *T. asiatica* (AB462829-AB462830) or *T. solium* (AB505026) was lower than that, 99.1-99.2% or 94.8%, respectively. These results strongly suggest that our sample is a hybrid between the 2 *Taenia* species.

Hybridization between *T. saginata* and *T. asiatica* was reported recently in Asian countries [5-8] but not yet in Cambodia. In this study, we performed an allelic analysis of a *Taenia* tapeworm strobila collected from a human to rule out the hybrid issue in Cambodia and confirmed that our specimen is a hybrid between *T. saginata* and *T. asiatica*.

Human taeniasis (by *T. solium*, *T. asiatica*, and *T. saginata*)

are known to be endemic in East and Southeast Asia, including Thailand, Vietnam, Lao PDR, which neighbor with Cambodia, and also in Nepal and India [8,11,14]. However, in Cambodia, reports of human taeniasis due to *T. asiatica* have not yet been documented. Our present finding implies the possible existence of *T. asiatica* in the northern part of Cambodia, although it remains to be confirmed by further studies.

The transmission of tapeworms is closely related to the lifestyle and cultural characteristics of people, which involve the consumption of undercooked meat infected with viable metacestodes (i.e., cysticerci) [14]. In Cambodia, there is a traditional food habit of eating cattle or pig meat, for example raw beef salad ('*pleah sach ko*'). However, as the intermediate host and life cycle of the hybrid between *T. saginata* and *T. asiatica* are unclear now, it is necessary to examine the metacestodes from domestic animals using genetic markers. Thus, the risk factors as well as the level of exposure of the people in Cambodia to the agents causing human taeniasis, including the hybrid, should be evaluated.

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## CONFLICT OF INTEREST

The authors declare no competing interests related to this study.

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